Title
Lexis and Grammar of Mitochondrial RNA Processing in Trypanosomes

Journal
Trends in Parasitology, 36(4)

ISSN
1471-4922

Authors
Aphasizheva, Inna
Alfonzo, Juan
Carnes, Jason
et al.

Publication Date
2020-04-01

DOI
10.1016/j.pt.2020.01.006

Peer reviewed

HHS Public Access

Author manuscript

Trends Parasitol. Author manuscript; available in PMC 2020 April 01.

Published in final edited form as:

Trends Parasitol. 2020 April ; 36(4): 337–355. doi:10.1016/j.pt.2020.01.006.

Lexis and Grammar of Mitochondrial RNA Processing in Trypanosomes

Inna Aphasizheva[1,*], **Juan Alfonzo**[2], **Jason Carnes**[3], **Igor Cestari**[4], **Jorge Cruz-Reyes**[5], **H. Ulrich Göringer**[6], **Stephen Hajduk**[7], **Julius Lukeš**[8], **Susan Madison-Antenucci**[9], **Dmitri A. Maslov**[10], **Suzanne M. McDermott**[3], **Torsten Ochsenreiter**[11], **Laurie K. Read**[12], **Reza Salavati**[4], **Achim Schnaufer**[13], **André Schneider**[14], **Larry Simpson**[15], **Kenneth Stuart**[3], **Vyacheslav Yurchenko**[16,19], **Z. Hong Zhou**[15], **Alena Zíková**[8], **Liye Zhang**[17], **Sara Zimmer**[18], **Ruslan Aphasizhev**[1]

[1]Department of Molecular and Cell Biology, Boston University Medical Campus, Boston, MA 02118, USA [2]Department of Microbiology, The Ohio State University, Columbus, OH 43210, USA [3]Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, WA 98109, USA [4]Institute of Parasitology, McGill University, 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, H9X3V9, Québec, Canada [5]Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843, USA [6]Department of Molecular Genetics, Darmstadt University of Technology, 64287 Darmstadt, Germany [7]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA [8]Institute of Parasitology, Biology Centre, Czech Academy of Sciences and Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic [9]Parasitology Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY 12201, USA [10]Department of Molecular, Cell, and Systems Biology, University of California – Riverside, Riverside, CA 92521, USA [11]Institute of Cell Biology, University of Bern, Baltzerstrasse 4, Bern CH-3012, Switzerland [12]Department of Microbiology and Immunology, University at Buffalo, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY 14203, USA [13]Institute of Immunology and Infection Research, University of Edinburgh, Edinburgh EH9 3FL, UK [14]Department of Chemistry and Biochemistry, University of Bern, Bern CH-3012, Switzerland [15]Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, CA90095, USA [16]Life Science Research Centre, Faculty of Science, University of Ostrava, Ostrava, Czech Republic [17]School of Life Science and Technology, ShanghaiTech University, Shanghai, 201210, China [18]University of Minnesota Medical School, Duluth campus, Duluth, MN 55812, USA [19]Martsinovsky Institute of Medical Parasitology, Sechenov University, Moscow, Russia

Abstract

Trypanosoma brucei spp. cause African human and animal trypanosomiasis, a burden on health and economy in Africa. These hemoflagellates are distinguished by a kinetoplast nucleoid containing mitochondrial DNAs of two kinds: maxicircles encoding ribosomal RNAs (rRNAs) and proteins and minicircles bearing guide RNAs (gRNAs) for mRNA editing. All RNAs are produced

[*]Correspondence: innaaf@bu.edu (I. Aphasizheva).

by a phage-type RNA polymerase as 3′ extended precursors, which undergo exonucleolytic trimming. Most pre-mRNAs proceed through 3′ adenylation, uridine insertion/deletion editing, and 3′ A/U-tailing. The rRNAs and gRNAs are 3′ uridylated. Historically, RNA editing has attracted major research effort, and recently essential pre-and postediting processing events have been discovered. Here, we classify the key players that transform primary transcripts into mature molecules and regulate their function and turnover.

Mitochondrial Gene Expression in Trypanosomes: A Trove of Unconventional Biology

Protist parasites of the genus *Trypanosoma* have occupied the research spotlight since 1895 when David Bruce identified *Trypanosoma brucei* as the causative agent of animal trypanosomiasis (Nagana), and later works linked these organisms to sleeping sickness in humans [1]. Biomedical, economic, and societal impact of parasite infections warranted in-depth studies of the fascinating biology underlying *T. brucei* metabolism, development, and interactions with the insect vector and mammalian host [2]. Among the most striking cellular features of these parasites is the bipartite mitochondrial genome consisting of **maxicircles** (see Glossary) and **minicircles**, and aggregately referred to as the **kinetoplast** DNA (kDNA). In *T. brucei*, maxicircles are catenated with minicircles into a single network and compacted by histone-like basic proteins. Maxicircles, an equivalent of mitochondrial genomes (mtDNA) in other organisms, encode 9S and 12S rRNAs, two ribosomal proteins [3], and 16 subunits of respiratory complexes. Unlike most organellar genomes, kDNA lacks tRNA genes [4,5], and 12 maxicircle genes are present as **cryptogenes** whose transcripts require RNA editing to restore a protein-coding capacity [6]. The editing is mediated by hundreds of **guide RNAs** (**gRNAs**) which are mostly encoded by minicircles, with only two gRNAs encoded by maxicircles. The distinct, albeit interlinked, maxicircle and minicircle genomes are transcribed independently, but the information converges at the post-transcriptional level whereby minicircle- encoded gRNAs direct editing of maxicircle encoded pre-mRNAs. The evolution of editing and whether this process confers a selective advantage to **kinetoplastids** remain the subject of a debate [7], but the existence of alternatively edited mRNA sequences and cognate gRNAs raises a possibility that editing-driven protein diversity may be functionally relevant [8–11]. Historically, much attention has been focused on the RNA-editing mechanism and composition of editing complexes [12–18] while more recently major advances have been made in understanding transcription [19], primary RNA nucleolytic processing [20–22], 5′ [19] and 3′ [23–28] modifications, and ribosome biogenesis and translation processes [3,29–31]. The perceived complexity of mitochondrial gene expression has been exacerbated by a recent influx of RNA-processing factors and numerous names often referring to the same entity. Bearing in mind that the functions of only a few proteins and complexes are established beyond reasonable doubt, we nonetheless submit that the process of discovering major players is close to completion. Here, we outline major stages in kinetoplast RNA processing (Figure 1) and build on previous attempts [32,33] to introduce a consensus nomenclature for respective protein and ribonucleoprotein (RNP) complexes, enzymes, and factors in *T. brucei*. Given that orthologs of nearly all *T. brucei* proteins listed in Table 1 (Key Table) are readily distinguishable in

related organisms, this nomenclature should be broadly applicable to other members of the Kinetoplastea.

Nucleolytic Processing of Primary Transcripts

In most organisms, primary polycistronic mtDNA transcripts are punctuated by tRNAs whose excision by RNases P and Z defines functional RNA boundaries. Although loss of mtDNA-encoded tRNA genes renders such a mechanism inapplicable to *T. brucei*, it has been held that mature mRNAs and rRNAs with uniformly monophosphorylated 5′ and well-demarcated 3′ termini reflect partitioning of a polycistronic precursor by an unknown endonuclease [34,35].To that end, a prominent transcription start site has been mapped within the maxicircle divergent region ~1200 nt upstream of 12S rRNA [36], and transcription proceeding through intergenic regions has been reported [37]. The uridylated rRNA [38] and adenylated mRNA [39] termini also typify distinct 3′ end modification mechanisms for these RNA classes. Conversely, short (30–60 nt) gRNAs maintain 5′ triphosphates characteristic of the transcription-incorporated initiating nucleoside triphosphate and, similar to rRNAs, are 3′ uridylated [40]. However, the only candidate gRNA precursor processing endonuclease KRPN1 (mRPN1) [41] is dispensable for axenically grown bloodstream stage [37]. Thus, it may be argued that the essentiality of RNA editing [42], which requires mature gRNAs [21,43], renders KRPN1 an unlikely contributor to gRNA precursor processing. The observations conducive to the endonucleolytic model have been re-examined in light of **mitochondrial 3′ processome (MPsome)** discovery [20]. These studies recognized the MPsome-catalyzed 3′–5′ exonucleolytic degradation as the major nucleolytic processing pathway for mRNA and rRNA [25], and gRNA [20] precursors. Composed of KRET1 **terminal uridyltransferase (TUTase)** [44], KDSS1 exonuclease [22], and mitochondrial processome subunits (MPSSs) 1–6 (Table 1), the purified MPsome displays 3′–5′ RNA degradation, RNA hydrolysis-driven double-stranded RNA unwinding, and 3′ RNA uridylation activities. Although the autonomous KDSS1 is inactive, incorporation into the MPsome converts this polypeptide into a highly processive exonuclease capable of degrading structured RNAs to 5–6 nt fragments. On the other hand, individual KRET1's robust UTP polymerization activity [45] is tamed upon MPsome assembly to adding 1–15 Us, a pattern consistent with the U-tails observed in steady-state RNA [11,46]. Cumulatively, detection of *in vivo* uridylated precursors and degradation intermediates [20,27], stimulation of *in organello* KRET1-dependent RNA decay by UTP [23], and MPsome's preference for U-tailed substrates suggest that uridylation by KRET1 activates RNA degradation by KDSS1. It is unclear whether substrate tunneling occurs within the same particle, but a coupling between RNA uridylation and degradation by 3'–5' **RNase II**/RNB-type exonuclease appears to be a highly conserved and phylogenetically widespread mode of RNA decay [47,48].

Exonucleolytic processing is often a case of regulated decay whereby mature 3′ termini are defined by a degradation blockade at a specific sequence, structure, or protein-binding site. In the *T. brucei* mitochondrion, antisense transcripts cause MPsome pausing at 10–12 nt before the double-stranded region at which point the MPsome-embedded KRET1 likely adds a U-tail causing disengagement from the substrate [19,20]. It follows that the precise transcription start site on the antisense strand defines the position of the mature 3′ terminus

of the sense transcript (Figure 2). The antisense model of gRNA 3′ end definition is consistent with bidirectional transcription from converging promoters otherwise recognized as imperfect 18-nt inverted repeats [49] that flank almost all gRNA genes in *T. brucei* minicircles [50,51]. Identification of gRNA-sized short antisense RNAs and accumulation of antisense precursors upon KRET1 and KDSS1 knockdowns [20] further indicate that sense and antisense precursors hybridize with their complementary 5′ regions. In the current model, the length of a double-stranded region, which is the distance between respective transcription start sites, likely defines gRNA length prior to uridylation [20]. However, most *T. brucei minicircles* encode 3 or 4 gRNA cassettes [51], and primary RNAs may exceed the linear length of a minicircle [20,21]. Hence, an extensive transcription of both strands likely generates much longer double-stranded RNAs that are degraded by an as yet unknown mechanism. Conversely, the *Leishmania tarentolae* minicircle typically contains a single gRNA gene and lacks recognizable inverted repeats [52]. Although both strands are transcribed [53], the gRNA-flanking sequences are dissimilar to those of *T. brucei*, which indicates a divergent nature of minicircle promoters among trypanosomatids. The maxicircle promoters remain to be identified, but detection of antisense transcription start sites near intergenic regions and the presence of corresponding noncoding antisense transcripts make a reasonable argument for a general mechanism of 3′ end definition for minicircle and maxicircle transcripts [19].

Modification of the 5′ End

The 5′ monophosphorylated termini of maxicircle-encoded rRNAs and mRNAs have long been interpreted as indicative of endonucleolytic partitioning of polycistronic precursors. It is, however, unfeasible to produce more than one monocistronic mRNA from a precursor by 3′–5′ degradation. This logic dictates that: (i) each gene rests under the control of a dedicated promoter; (ii) the 5′ terminus is set by transcription initiation; (iii) inorganic pyrophosphate (PPi) is selectively removed from initiating nucleoside triphosphate in mRNAs and rRNAs, but not in gRNAs; and (iv) transcription may proceed across multiple genes and produce a 3′ extended precursor of which only the most 5′ coding region is preserved after 3′–5′ trimming. Identification of the **5′ pyrophosphate processome (PPsome)** complex partially resolved the question of differential phosphorylation status and linked 5′ PPi removal to mRNA stability [19]. A stable protein complex of MERS1 NUDIX [nucleoside diphosphate linked to (X)] hydrolase and MERS2 **PPR** (**pentatricopeptide repeat**) RNA binding subunit, the MPsome selectively binds to degenerate G-rich motifs found near mRNA 5′ ends, but not in gRNAs. MERS1 hydrolase is catalytically inactive as an individual protein while MERS2 apparently confers both binding specificity and affinity for RNA substrate. Remarkably, MERS1 downregulation or replacement with an inactive version effectively eliminates most mRNAs but exerts negligible effects on gRNAs and rRNAs. It appears that rRNA is stabilized by different factors, possibly those involved in ribosome biogenesis [31]. Although PPsome-dependent mRNA protection against 3′–5′ degradation (see below) and the essential role of PPi removal are evident, the mechanistic insights into these processes will likely come from understanding PPsome's interactions with **RNA-editing substrate-binding complex** (**RESC**) and **kinetoplast polyadenylation complex** (**KPAC**) discussed below [28].

Modifications of the 3′ End

Nontemplated 3′ nucleotide additions often wield profound influence on RNA processing, function, trafficking, and turnover [54]. In *T. brucei*, mitochondrial RNA 3′ modifications can be categorized into U-tailing by KRET1 TUTase (gRNAs and rRNAs), A-tailing by KPAP1 poly(A) polymerase (most mRNAs [27]), and A/U-extensions which require both enzymes and a complex of kinetoplast polyadenylation factors 1 and 2 (KPAF1/2, [26]). Lack of pronounced RNA substrate specificity for KRET1 and KPAP1 raises the question of accessory factors that enable modifications of distinct RNA classes, and the functionality of these extensions. The presence of U-tails in gRNAs and rRNAs, as well as nontemplated uridine residues sometimes found in mRNAs between the 3′ untranslated region (UTR) and the A-tail [26], indicates that uridylation by the MPsome-embedded KRET1 is a default post-trimming 3′ modification. It is plausible that U-tailing causes the MPsome to disengage from the precursor when degradation pauses near a double-stranded region formed by antisense RNA. However, the U-tail itself does not exert an appreciable impact on mature gRNA or rRNA stability [21] and its functionality beyond termination of processing remains debatable. Conversely, a short (15–30 nt) A-tail decorates most mRNAs and impacts stability depending on the transcript's editing status [24,27,55]. As demonstrated by KPAP1 poly(A) polymerase loss-of-function studies [25,27] and *in organello* decay assays [24], adenylation mildly destabilizes **pre-edited** transcripts only to become essential for maintaining RNAs that are edited beyond initial **editing sites** at the 3′ end. A short A-tail also stabilizes **never-edited mRNAs** (those that contain an encoded open reading frame and do not require editing). The coupling between an mRNA's editing status and opposing effects of adenylation points toward a surveillance system capable of both sensing the progression of internal **U-insertions/deletions** and enabling 3′ A-tail addition and stabilizing function. In molecular terms, sequence-specific activators and inhibitors would be expected to modulate mRNA adenylation by KPAP1, and the resistance of such a modified molecule to decay by the MPsome. The respective functions have been attributed to KPAF3 [25] and KPAF4 [28], which belong to a family of pentatricopeptide (35 amino acid) repeat-containing RNA binding (PPR) proteins. Discovered in land plants [56], the helix–turn–helix PPR motif recognizes a single nucleoside via side chains occupying cardinal positions 5 and 35 of the repeat (or the last position in a longer structure). An array of adjacent PPR repeats often folds into a superhelical domain capable of binding to a specific RNA sequence and recruiting or blocking various enzymes [57–59]. In this context, KPAF3 reportedly binds to G-rich pre-edited mRNAs with sufficient affinity and coverage to stabilize these species [25]. *In vitro* reconstitution experiments demonstrate that KPAF3 stimulates KPAP1 polyadenylation activity and this effect depends on the presence of the G-rich site near the 3′ end. Remarkably, KPAF3 binding is eliminated by the initiating editing events leaving the stability of edited RNA reliant on the A-tail added prior to editing [25]. Thus, KPAF3 functions as editing sensor and bona fide polyadenylation factor thereby connecting the internal sequence changes and 3′ adenylation [25–27].

The most apparent A-tail function would be protecting mRNA against degradation by the MPsome. However, *in vitro* studies show that A-tailed RNAs can be degraded by the purified MPsome, albeit less efficiently than uridylated substrates [20]. The A-tailed

partially edited pre-mRNAs are also somehow prevented from the addition of the 200–300 nt A/U-tail. This modification marks a **fully edited** molecule [27] and channels translationally competent mRNA for translation [26,29,30]. Finally, the mechanism of mRNA stabilization by the PPsome must reconcile predominant binding of this complex to the 5′ end with blocking 3′–5′ degradation [19]. To rationalize these observations, Mesitov *et al.* envisioned a trans-acting factor that recognizes a nascent A-tail and facilitates an interaction between PPsome occupying the 5′ end and polyadenylation complex (KPAC) bound to the 3′ end [28]. It has been proposed that the resultant circularization increases mRNA resistance to degradation and uridylation, and, therefore, to premature A/U-tailing and translational activation of partially edited transcripts [28]. Trypanosomal genomes apparently lack mitochondrially targeted canonical RRM motif-containing poly(A) binding protein, but such function is fulfilled by KPAF4. This PPR protein is almost entirely composed of seven repeats of which five are predicted to bind sequential adenosine residues [60]. Copurification studies support KPAF4 interactions with KPAC components (KPAP1, KPAF1/2) and RESC-mediated proximity with the PPsome. Accordingly, the A-tail has been identified as the predominant *in vivo* binding site while *in vitro* KPAF4 selectively recognizes adenylated substrates. Indeed, KPAF4 renders adenylated RNA more resistant to degradation by the MPsome and uridylation by KRET1 TUTase *in vitro* [28].

Although direct demonstration of mRNA circularization is lacking, this event can be imagined as a quality check point to ensure 5′ end occupancy by the PPsome and correct termination of 3′–5′ trimming downstream from the KPAF3 binding site. In this scenario, KPAF3 binding likely selects a correct 3′ UTR among trimmed isoforms and stimulates polyadenylation of eligible precursor by KPAP1. KPAF4 binding to a nascent A-tail may then enable interaction with the 5′ end-bound PPsome, hence stimulating mRNA circularization. Consequentially, only A-tailed mRNAs would proceed through the editing cascade while the variants truncated beyond KPAF3 binding sites become uridylated and degraded [25]. It follows that upon editing completion at the 5′ end, a signaling event takes place to disrupt circularization and enable access of KPAF1/2 factors and KRET1 TUTase, thus extending the pre-existing short A-tail into the long A/U-tail. Although these inferences require further testing, it seems plausible that PPsome displacement from the 5′ end by final editing acts may constitute this signaling event, at least in the case of pan-edited mRNAs (Figure 3).

U-insertion/Deletion mRNA Editing

Editing Process

In *T. brucei*, six of the 18 annotated mRNAs encode predicted polypeptides while the remaining 12 transcripts undergo editing to acquire a protein-coding sequence. The extent of editing varies from minor, typified by insertion of four Us into three closely spaced sites (COII mRNA, [6]), to moderate (e.g., *cyb* mRNA, 34 Us are inserted into a confined region near the 5′ end [61]), to **pan-editing** during which hundreds of uridines are inserted or deleted throughout the entire transcript (e.g., ND7 mRNA [62]). The determinants of position-specific U-insertions and deletions were discovered in the Simpson laboratory as short patches of complementarity between edited mRNA and maxicircle DNA in *L.*

tarentolae [40]. By allowing for G–U, in addition to canonical Watson–Crick base-pairing, short (30–60 nt) RNAs transcribed from minicircles have been recognized as carriers of genetic information and termed guide RNAs. *In vitro* experiments by the Stuart laboratory directly demonstrated that gRNAs indeed constitute the necessary and sufficient source of editing information [63–65]. Although the first gRNAs were discovered in the maxicircle, further work in *Leishmania* established that most gRNAs are encoded in minicircles [66]. In *T. brucei*, only two gRNAs have been identified in the maxicircle: a *cis*-acting element embedded into the 3′ UTR of COII mRNA [67], and a *trans*-acting gRNA that completes editing of the MURF2 mRNA. The secondary structure of gRNA–mRNA dictates the editing site selection and the extent of U-insertions and deletions [63] (Figure 4). The initial gRNA–mRNA interaction is accomplished via a short (10–12 nt) region of complementarity between the gRNA's 5′ **anchor** region and the pre-edited mRNA. The remaining guiding segment forms an imperfect duplex with pre-mRNA resulting in looping out of single-stranded uridines in mRNA (deletion sites) or purine nucleotides in gRNA (insertion sites). At either site, the mRNA is cleaved at the first unpaired nucleotide adjacent to the 5′ anchor duplex. The resultant structures of deletion and insertion intermediates are distinct: single-stranded uridines become exposed to a 3′–5′ exonucleolytic attack in the former, while a single-stranded gap is created between two helices in the latter. Upon trimming single-stranded uridines from the **5′ cleavage fragment** in the deletion site or adding a gap-specified number of Us into the insertion site, the fragments are joined to restore mRNA continuity. Both types of sequence changes extend the double-stranded anchor region. Pan-editing requires multiple overlapping gRNAs, and there is a method to it: sequence changes directed by the initiating gRNA create a binding site for the next one to ensure an overall 3′–5′ polarity along the **editing domain**. However, editing may not always proceed strictly 3′ to 5′ as 'mis-edited' **junctions** are present at the leading edge of editing in the majority of steady-state mRNAs [53,68–70]. The role of junctions is not understood, but they likely represent a mixture of regions that undergo re-editing to canonical edited sequence, dead-end products, or mRNAs with alternative noncanonical coding sequences [18].

A single editing domain may cover an entire mRNA [71], or an isolated region [62]. An individual gRNA can theoretically direct insertions and deletions at several closely spaced sites (**editing block**), but, as editing progresses within the block, the interaction between gRNA and mRNA 5′ cleavage fragment is supported by fewer base pairs. Stabilizing the 5′ cleavage fragment–mRNA tethering by additional base pairing stimulates cleavage and the full editing cycle *in vitro* [72–74], but it is unclear how the problem of editing at distal sites within one block or across sequential blocks is solved *in vivo*. An active displacement of a gRNA with diminishing '3′ tether' by **RNA helicase** is among possible solutions that would enable binding of succeeding gRNA within a domain.

Editing Reactions

Editing reactions are catalyzed by enzymes embedded in the ~20S (~800 kDa) **RNA-editing catalytic complex** (**RECC**), a remarkable example of a modular assembly that enables broad functionality on distinct RNA substrates [75–79] (Figure 4). A common core particle consists of U-insertion (KRET2 TUTase, KREPA1 zinc-finger protein, and KREL2 RNA ligase) and U-deletion (KREX2 exonuclease, KREPA2 zinc-finger protein, and KREL1

RNA ligase) subcomplexes, and six structural and/or RNA-binding proteins (KREPA3, KREPA4, KREPA5, KREPA6, KREPB4, and KREPB5). The U-insertion and U-deletion subcomplexes likely function independently while most of the remaining components are essential for assembly and/or integrity of the entire core particle [13]. The core particle is shared among at least three RECC isoforms distinguished by association with endonuclease modules. Each module is composed of an **RNase III** endonuclease and a partner protein(s) and is primarily responsible for recognition and cleavage of insertion and deletion sites. The U-deletion sites are recognized by the RECC isoform with KREN1$_+$KREPB8$_+$KREX1, while U-insertion sites are recognized by the RECC isoforms with KREN2$_+$KREPB7 or KREN3$_+$KREPB6, which display distinct and overlapping specificities [80–84]. The canonical RNase III catalytic domain typically forms a functional homodimer with two active sites that introduce four cuts into both strands of a double-stranded RNA [85]. By contrast, editing endonucleases appear to cleave only mRNA. It seems plausible that RNA hydrolysis is restricted to a single cut by heterodimer formation between KREN1, KREN2, or KREN3, and catalytically inactive degenerate RNase III domains in KREPB4 or KREPB5 [86]. A contribution of RNase III partner proteins KREPB8, KREPB7, or KREPB6 to modulating cleavage activity is also possible [87,88]. Crosslinking mass-spectrometry points to interactions involving RNase III domain dimerization between editing endonucleases with partner proteins KREPB6, B7, or B8, and core proteins KREPB4 and KREPB5 [87–89]. Binding of KREN1, KREN2, and KREN3 modules to a common core containing U-deletion, U-insertion, and ligase activities highlights RECC's modular nature, but the nature of interactions responsible for mutually exclusive contacts between the core and distinct modules remains unclear.

Within the common core, the U-deletion and U-insertion cascades are spatially separated by virtue of editing enzymes binding to zinc-finger proteins, KREPA2 and KREPA1, respectively [90–93]. KREX1 and KREX2 proteins possess **exonuclease–endonuclease–phosphatase (EEP)** catalytic domains and display single-stranded uridine-specific 3′–5′ exonuclease activity *in vitro* [92,94]. However, their protein–protein interactions are remarkably distinctive: the essential KREX1 belongs to the KREN1 endonuclease module, and is responsible for the main U-deletion activity; the dispensable KREX2 probably represents a structural component of the U-deletion subcomplex [81,89]. Fittingly, *L. tarentolae* KREX2 lacks a catalytic domain but remains associated with the U-deletion subcomplex [75]. In the U-insertion subcomplex, KRET2 TUTase binds to KREPA1, which results in a mutual stabilization and stimulation of TUTase activity [43,89,95–97]. Selectivity of uridine incorporation is determined by KRET2's intrinsic specificity for UTP [98] rather than the nature of the opposing nucleotide in the gRNA. To that end, the +1U addition occurs equally efficient irrespective of the corresponding nucleotide in gRNA, but the +2U addition occurs only if the +1U forms a base pair with either adenosine or guanine. Consequentially, both purine bases in guiding positions direct U-insertions with similar efficiency [72,95]. RNA editing ligases 1 and 2 (KREL1 and KREL2) have been identified as components of U-deletion and U-insertion subcomplexes, respectively [89–91]. Although spatial separation appears to suggest specialized roles, only KREL1, but not KREL2, is essential for cell viability [42,99,100].

Editosome Definition

From the early reports of RNA ligase-containing complexes sedimenting in glycerol density gradients as particles with apparent 20S to 50S values [101,102], the quest for an elusive 'editosome' evolved into a concept of an **RNA-editing holoenzyme**. For the purposes of this review, we shall equate the editosome and editing holoenzyme and define this entity as an RNA-mediated assembly of the RECC, RESC, and **RNA-editing helicase REH2 (REH2C)** complexes. It is a virtual certainty that additional components are also involved in editosome functioning [15,17,103]. This definition stems from parallel lines of inquiry by the Stuart, Aphasizhev, and Lukeš laboratories that identified an ~800 kDa protein complex (originally termed mitochondrial RNA-binding complex 1, MRB1, and gRNA-binding complex, GRBC), of which two components are essential for gRNA stability [104–106]. Originally named GRBC1 and GRBC2, these homologous polypeptides lack annotated motifs and similarity to any protein outside of kinetoplastids [63]. GRBC1 and GRBC2, also referred to as GAP2 and GAP1, respectively [106], form a stable heterotetramer which binds gRNA *in vitro* and *in vivo* [46]. Extensive copurification and yeast two-hybrid screens further dissected MRB1 into two relatively stable protein complexes: a ~20-component RESC complex, which includes RESC1 (GRBC1, GAP2) and RESC2 (GRBC2, GAP1), and three-subunit REH2C (Table 1). It appears that both RESC and REH2C bind editing substrates, intermediates, and products, and engage in RNA mediated interactions with the catalytic RECC complex [46,107–110]. All but five of the RESC subunits lack discernible motifs or similarities to non-kinetoplastid proteins, although several exhibit *in vitro* RNA-binding activity [15,17,103]. Most subunits are essential for cell viability, and their knockdowns produce phenotypes consistent with an inhibited editing process. Recently, substantial progress has been made in deciphering roles of individual factors. The RESC1/2 tetramer appears to be solely responsible for gRNA stabilization [105,106]. Deep sequencing studies showed that the RRM/RGG-containing RESC13 (RGG2) and proximal protein RESC11A (MRB8180) contribute to editing processivity within an extended domain [69,111]. These two proteins promote the formation of junctions, implying a critical role of these regions in editing progression [69]. Conversely, the product of a duplicate gene RESC12A (MRB8170) has been implicated in editing initiation and in constraining the region of active editing [69,112]. Biochemical attempts to refine RESC architecture indicate a modular organization with potential protein clusters responsible for interaction with the RECC and polyadenylation complexes [46,113]. However, an unambiguous assignment of specific polypeptides to functional modules awaits elucidation of a high-resolution structure of the RESC complex.

RNA editing is an essential processing step for a subset of mitochondrial transcripts and must be integrated into a general pathway of producing translation-competent mRNAs. Accumulating evidence suggests that the RESC is responsible for coordinating pre- and postediting processing events via RNA-mediated contacts with 5′ and 3′ modification complexes, and auxiliary factors. Furthermore, the catalytic RECC isoforms appear to act on RESC-bound editing substrates in a transient manner. The RNA-mediated interaction between RESC and PPsome has been deduced from copurification of RESC1/2 (GRBC1/2) and MERS1 hydrolase [19,105,114] whereas *in vivo* proximity biotinylation identified RESC19 (MERS3) as the most plausible adapter the for RESC-PPsome contact [19]. An

independent study predicted Z-DNA-binding domains in RESC19 (termed RBP7910) and showed that *in vitro* this protein preferentially binds RNAs containing poly(U) and poly(A/U)-rich sequences [115]. Likewise, KPAC components have been consistently detected in various RESC preparations and particularly in those with tagged RESC15–18 [25,28,46]. Furthermore, RESC13 (RGG2) and surrounding proteins likely mediate contacts between RESC and RECC complexes [46]. Finally, RNA-editing helicase 2 (KREH2, see below) preferentially associates with RESC variants purified by tagging of either RESC1 or RESC2 [46,105,116–118]. Thus, the RESC complex functions not only in binding of RNA editing substrates, intermediates, and products, but also recruits mRNA 5′ and 3′ modification complexes, and auxiliary factors.

The KREH2 complex, termed REH2C, consists of DEAH/RHA RNA helicase KREH2, and KREH2-associated factors 1 (KH2F1) and 2 (KH2F2). KH2F1contains eight C2H2 zinc fingers while KH2F2 lacks any identifiable motifs [119] (Table 1). Isolated REH2C exhibits ATP-dependent 3′–5′ dsRNA unwinding activity and cosediments with a major peak of same activity in mitochondrial extracts [118]. Zinc-finger protein KH2F1 emerged as an adaptor connecting KREH2 helicase with the RESC while gRNA–mRNA hybridization has also been implicated in facilitating this interaction [119]. KREH2 and KH2F1 knockdowns display consistent phenotypes of increased editing pausing and reduced processivity of editing, which are indicative of REH2C participation in editosome remodeling [107,119]. It must be emphasized that the reciprocal affinity purifications remain the most salient evidence of the editosome being an RNA-based assembly of RECC, RESC, and REH2C protein complexes [104–106,117,118].

Auxiliary Factors

MEAT1 TUTase

Mitochondrial editing-like complex-associated TUTase 1 (MEAT1) has been identified by homology to KRET1 and KRET2 and displays an exquisite UTP specificity *in vitro* and the ability to incorporate uridines into double-stranded substrates imitating U-insertion editing sites [120,121]. In mitochondrial extracts, this enzyme interacts with RECC variant missing the entire U-insertion subcomplex (KRET2, KREPA1, and KREL2), but MEAT1 is not detectable in most RECC preparations. MEAT1 RNAi knockdown does not appreciably inhibit RNA editing, which leaves the *in vivo* RNA substrates and function of this enigmatic enzyme to be elucidated.

Putative Poly(A) Polymerase KPAP2

A putative kinetoplast poly(A) polymerase KPAP2 has been identified by homology to the human mitochondrial enzyme and apparently is not required for axenic *T. brucei* growth in either bloodstream or procyclic life stages [122]. Although the KPAP2 protein sequence is highly similar to that of KPAP1 its enzymatic identity and function remain to be established. Available proteomics data do not support KPAP2 association with KPAC [25,27,28].

KREH1 RNA Helicase

Editing reactions are expected to produce an mRNA–gRNA duplex wherein gRNA must be eventually displaced to allow binding of a sequential gRNA, or before the edited transcript can be translated. It stands to reason that active remodelers, such as DEAD/H-box RNA helicases, would be involved, and indeed two such proteins have been implicated in the editing process. However, dissecting their specific roles, RNA targets, and mechanism of action proved to be challenging. Knockdown of KREH1 (Hel61) helicase [123] affected editing mediated by two or more overlapping gRNAs [124] but mechanistic placement of KREH1 into an mRNA editing or processing context requires further investigation of its interactome and impacts on editing.

RNA-Binding Factors

Kinetoplast mitochondrial RNA-binding proteins 1 and 2 (KMRP1 and 2), originally called gBP21 and gBR25, then MRP1 and MRP2, were identified independently in *T. brucei* by UV-induced crosslinking with synthetic gRNA (gBP21, [125]), in *Crithidia fasciculata* as poly(U) binding proteins (gBP21 and gBP25, [126]), and in *L. tarentolae* via crosslinking to double-stranded RNA resembling the U-deletion site (MRP1 and MRP2 [114]). Extensive biochemical and structural studies concluded that KMRP1 and KMRP2 assemble into a ~100 kDa heterotetramer, which binds both single- and double-stranded RNAs with high affinity [114,127,128]. These RNA-binding properties are manifested by an RNA-annealing activity, an attractive accessory function that may promote gRNA binding to cognate mRNA targets [129,130]. However, the transcript-specific impact of dual KMRP1/2 repression suggests a contribution to stabilization of moderately edited and some never-edited mRNAs rather than direct participation in the editing process. In support of this notion, RNAi experiments demonstrated that MRP1/2 depletion virtually eliminates the edited form of the **moderately edited** *cyb* mRNA, but exerts little impact on the pre-edited transcript [131–133]. While much is known about the KMRP1/2 structure and *in vitro* properties, the definitive function of this RNA-binding complex remains to be established. Much of the same narrative applies to KRBP16 (RBP16), which carries N terminal cold-shock and C terminal RG-rich domains [134]. RNAi studies revealed an overlap between mRNA sets negatively affected by individual KMRP1/2 and KRBP16 knockdowns: edited *cyb* mRNA, but not any other edited transcripts, was severely downregulated, while never-edited CO1 and ND4 transcripts also declined [132]. KRBP16 *in vitro* properties, such as RNA-binding affinity, RNA-annealing activity, and stimulation of editing activity, and the impact of RNAi knockdown on the initiation of *cyb* mRNA editing [131], are consistent with participation in the editing process, although the mechanistic role remains to be firmly established [135–138]. Another enigmatic RNA-binding protein, KRGG1, was serendipitously discovered in a large (>50S) RNP of unknown nature [139], and subsequently demonstrated to associate with the RESC complex [106,140]. A different study identified a ribosome-bound KRGG1 fraction, which would explain the observed sedimentation patterns, but found no impact on RNA editing [46]. Another arginine-glycine-rich protein KRGG3, originally identified by association with RESC1/2 proteins and termed MRB1820 [113], is essential for parasite viability [141]. However, most of KRGG3's interactions appear to be RNA-mediated while the RNAi knockdown does not significantly impact major mitochondrial RNA classes. A

structural study identified an ABC–ATPase fold and potential RNA-binding surface in KRBP72, initially termed MRB1590 [142]. KRBP72 knockdown specifically impacts editing of A6 mRNA [142]; however, an unequivocal functional placement of this factor also requires further investigation. Finally, participation of KREAP1 in editing [143,144] has been contravened by a report of general mitochondrial RNA upregulation upon its knockdown and nonessentiality for survival [145]. Overall, mitochondrial RNA-binding proteins are abundant and notoriously promiscuous in their interactions and pleiotropic effects on RNA steady-state levels [146], which makes an unequivocal definition of their function a challenge worth meeting.

Ribonucleases

Mitochondrial RNA processing most likely involves nucleolytic events beyond mRNA cleavage by editing KREN1, 2, and 3 endonucleases, KREX1 exonuclease, and 3′–5′ degradation by the MPsome-embedded KDSS1. To that end, three distinct enzymes have been identified and characterized to various degrees. The single-strand uridine-specific KRND1 3′–5′ exonuclease [147] displays *in vitro* specificity for single-stranded uridines, similar to that of KREX1 editing enzyme [4,92], and yet possesses an RNase D rather than EEP exonuclease domain. Given the diversity of U-tailed RNAs in the kinetoplastid mitochondrion, it is tempting to speculate on KRND1 involvement in regulating the 3′ modification state, but its definitive function remains to be established. The same narrative applies to KRPN1, an RNase III endonuclease with a characteristic double-stranded RNA-cleaving activity suggested to function in gRNA processing [37,41]. Further studies are required to reconcile KRPN1 RNA substrate specificity with an exonucleolytic mechanism of gRNA precursor processing by the MPsome. Finally, the discovery of PPR-repeat-containing proteinaceous RNase P (PROPR2 [148] (renamed here as KRNP1) supported earlier reports of RNase P activity-like which removes the 5′ leader from a synthetic tRNA precursor in mitochondrial lysate [149]. However, tRNAs are apparently imported into the mitochondrion with 5′ and 3′ extensions removed [150,151], which leaves the nature of KRNP1 *in vivo* substrates open to future inquiry.

Concluding Remarks

This review compiles 74 processing enzymes, RNA-binding proteins, and factors with unknown functionality that nonetheless are associated with RNA-processing complexes. Proteomics and interactions analyses allowed clustering most of these into five or six macromolecular assemblies, albeit with various degree of confidence. Although the list is almost certainly incomplete, with complexes and individual proteins, and their interactions and functions are constantly being reexamined, the overall flow of RNA processing in the trypanosome mitochondrion is taking shape and meaning. The key players responsible for maturation of 5′ and 3′ termini have been defined and initial insights gained into the molecular mechanism of internal sequence changes by editing. At this point, we suggest that the RNA-editing holoenzyme (editosome) represents an RNP that chiefly includes three relatively stable protein complexes (RECC, RESC, and REH2C) and RNA-editing substrates, intermediates, and products. It is understood that the definition of a protein complex is often a matter of purification technique and we posit that future structural studies

will shed light on stoichiometry and functions of individual subunits and modules. Because of sequence changes introduced by editing during mRNA processing, the overall picture is emerging of the mRNA fate being dictated by diverse PPR RNA surveillance factors. These proteins direct 5′ pyrophosphate removal, transcript stabilization and pre-editing A-tailing, monitor initiation and progression of editing, and signal its completion by stimulating the A/U-tailing. Displacement of bound PPRs from pre-edited mRNA by the editing process emerges as the principal quality-control mechanism. It remains to be established whether active RNP remodeling takes place or sequence changes alone suffice for this purpose. In any event, the plurality of PPRs and their capacity to read linear sequences and modulate the activity of RNA modification and degradation enzymes position this protein family as the focal point of mitochondrial RNA processing.

Moving forward, it is critical to map maxicircle promoters and determine the composition of transcription complexes acting on maxicircle and minicircle genomes. The timing and mutual dependence of mRNA synthesis and processing events, and the mechanism of 5′ to 3′ communication, will need to be addressed by a combination of molecular and imaging techniques. These insights will ultimately contribute to understanding the stage-specific nature of mitochondrial RNA processing events and their coordination with nuclear gene activity. Accumulating evidence points to the existence of alternatively edited mRNAs and highlights the questions of whether these are translated into proteins with distinct functions and how the mitochondrial ribosome selects edited mRNAs and recognizes the correct reading frame (see Outstanding Questions).

Acknowledgments

Aphasizheva lab is supported by NIH grant AI113157. Aphasizhev lab is supported by NIH grants AI091914 and AI101057. Cruz-Reyes lab is supported by NSF grant 1616845 and TAMU X-grant and T3 award. Göringer lab is supported by German Research Council (SPP1857). Lukeš, Yurchenko and Zíková labs are supported by the Czech Ministry of Education (OPVVV16_019/0000759), ERC CZ LL1601, and Czech Grant Agency grants 18-17529S and 18-15962S. Read lab is supported by NIH grants AI061580, GM129041, and AI139448. Hajduk lab is supported by NIH grant AI125487. Schnaufer lab is supported by MRC Senior Non-Clinical Fellowship MR/L019701/1. Schneider lab is supported by the NCCR grant “RNA & Disease” and by the grant 175563 from the Swiss National Science Foundation. Stuart lab is supported by NIH grant 5R01AI014102-43. Zimmer lab is supported by NIH grant AI135885.

Glossary

5′ and 3′ cleavage fragments
mRNA fragments generated by gRNA-directed endonucleolytic cleavage

5′ pyrophosphate processome (PPsome)
protein complex containing MERS1 NUDIX pyrophosphohydrolase and MERS2 PPR RNA-binding protein

Anchor
5′ part of the gRNA that forms a continuous 10–15 nt duplex with pre-edited, partially edited or fully edited mRNA; this region is responsible for initial gRNA–mRNA interaction

Cryptogene

a maxicircle gene with defective coding sequence; the defects are corrected by U-insertion/deletion editing with concomitant restoration of protein reading frame

Editing block
an mRNA segment covered by a single gRNA; often contains both U-insertion and U-deletion sites

Editing domain
an mRNA region covered by single or multiple overlapping gRNAs. In a multi-gRNA domain sequence changes directed by the initiating gRNA create the binding site for the subsequent one. The hierarchical gRNA binding provides for the overall 3′–5′ progression of editing events within a domain

Editing site
position of the gRNA-directed internal cleavage where uridines are either deleted from or inserted into the mRNA

EEP domain
endonuclease–exonuclease–phosphatase (EEP) domain in U-specific editing exonucleases

Fully edited mRNA
a final editing product; it contains a protein-coding frame

Guide RNA (gRNA)
a small noncoding RNA that specifies positions and extent of U-insertions and deletions by forming an imperfect duplex with pre-edited or partially edited mRNA. gRNA is typically 30–60 nucleotides (nt) in length and possesses a 5′ triphosphate and a 1–20 nt 3′ U-tail

Junction
a region present in most partially edited mRNAs at the 5′ leading edge of editing; often displays mis-edited and noncanonically edited sequences. Junction-containing transcripts may represent intermediates that will be reedited to canonical sequence, dead-end by-products, and mRNAs with a noncanonical protein-coding sequence

Kinetoplast
a densely packed nucleoprotein structure, disc-shaped and catenated in trypanosomatids, and dispersed to various degrees in most bodonids, that encloses mitochondrial DNA (kinetoplast DNA; kDNA). A nondividing *T. brucei* cell contains a single mitochondrion with a single kinetoplast composed of catenated maxicircles (~23 kb each, few dozen copies) and minicircles (~1 kb each, ~5000 units)

Kinetoplast polyadenylation complex (KPAC)
a ribonucleoprotein complex of KPAP1 poly(A) polymerase, and pentatricopeptide repeat-containing (PPR) RNA-binding proteins designated kinetoplast polyadenylation factors 1, 2, 3, and 4 (KPAF1, 2, 3, and 4)

Kinetoplastids

(class Kinetoplastea) flagellated protists characterized by the presence of a kinetoplast. Phylogenetically positioned within the phylum Euglenozoa, this group includes the obligatory parasitic trypanosomatids (family Trypanosomatidae), free-living and parasitic bodonids, and more distantly related taxa

Maxicircle
an equivalent of a typical mitochondrial genome; it includes a conserved ~15 kb region encoding 9S and 12S rRNAs, two gRNAs, and 18 protein genes. A variable region composed of repeated DNA sequences constitutes the rest of the molecule

Minicircle
the molecules forming the bulk of the kinetoplast. Approximately 400 sequence classes present at various frequencies encode ~930 gRNAs required for the editing process and 370 gRNA-like molecules that likely participate in gRNA processing

Mitochondrial 3′ processome (MPsome)
a protein complex composed of kinetoplast RNA-editing TUTase 1 (KRET1), 3′–5′ exonuclease KDSS1, and MPSS1–6 subunits lacking recognizable motifs

Moderately edited mRNA
a transcript with a few editing sites confined to a limited mRNA region.

Never-edited mRNA
a maxicircle transcript containing an encoded open reading frame which does not require editing

Pan-edited mRNA
a transcript that undergoes massive editing directed by multiple gRNAs. There can be two editing domains within a pan-edited mRNA

Partially edited mRNA
an intermediate of the editing process. Partially edited mRNAs often contain junctions whose sequences match neither pre-edited nor canonical fully edited mRNAs

PPR
pentatricopeptide (35 amino acids) helix–turn–helix repeat. PPR arrays are present in many trypanosomal mitochondrial RNA-binding proteins

Pre-edited mRNA
a 3′ processed monocistronic cryptogene transcript that must undergo editing to acquire an open reading frame and/or translation initiation and termination signals

RNA-editing catalytic complex (RECC)
formerly called ~20S editosome or RNA-editing core complex. A protein complex of 14 or more subunits, depending on the isoform; it includes pre-mRNA cleavage, U-insertion, U-deletion, and RNA-ligation enzymes, and structural and RNA-binding factors

RNA-editing helicase 2 complex (REH2C)

a protein complex formed by an ATP-dependent DEAH/RHA RNA helicase KREH2, zinc-finger protein KH2F1, and KH2F2 factor which lacks recognizable motifs

RNA-editing holoenzyme (editosome)
a ribonucleoprotein particle consisting of RECC, RESC, and REH2C complexes, and several auxiliary factors

RNA-editing substrate-binding complex (RESC)
formerly called mitochondrial RNA-binding complex 1 (MRB1) and gRNA-binding complex (GRBC). A ~20-subunit modular protein complex that likely exists in several isoforms; most components lack recognizable motifs. RESC binds RNA-editing substrates, intermediates, and products, and coordinates interactions of gRNA and mRNA with RECC, REH2C, and other auxiliary factors during editing. RESC has also been implicated in coordination of pre-mRNA 5′ and 3′ modification processes

RNA helicase
a motor protein capable of harnessing the energy from NTP hydrolysis to unwind double-stranded RNAs or to remodel ribonucleoprotein complexes

RNase II
exoribonuclease II cleaves single-stranded RNA in the 3’-to-5-direction yielding nucleoside 5′ monophosphates

RNase III
endoribonuclease III typically cleaves both strands in double-stranded RNA, leaving 5′ monophosphate and 3′ hydroxyl groups. RNase III-editing endonucleases cleave only the mRNA strand at an unpaired nucleotide adjacent to a gRNA–mRNA duplex

Terminal uridyltransferase (TUTase)
UTP-specific nucleotidyl transferase which adds U-residues to the 3′ end of RNA

U-insertion/deletion mRNA editing
a process by which U-residues are inserted into, or deleted from, a cryptogene transcript. Editing is directed by gRNAs and catalyzed by the RNA-editing holoenzyme (editosome)

References

1. Steverding D (2008) The history of African trypanosomiasis. Parasit. Vectors 1, 3 [PubMed: 18275594]
2. Maslov DA et al. (2019) Recent advances in trypanosomatid research: genome organization, expression, metabolism, taxonomy and evolution. Parasitology 146, 1–27 [PubMed: 29898792]
3. Ramrath DJF et al. (2018) Evolutionary shift toward protein based architecture in trypanosomal mitochondrial ribosomes. Science 362, eaau7735 [PubMed: 30213880]
4. Simpson AM et al. (1989) Kinetoplastid mitochondria contain functional tRNAs which are encoded in nuclear DNA and also small minicircle and maxicircle transcripts of unknown function. Nucleic Acids Res. 17, 5427–5445 [PubMed: 2762144]
5. Hancock K and Hajduk SL (1990) The mitochondrial tRNAs of *Trypanosoma brucei* are nuclear encoded. J. Biol. Chem 265, 19208–19215 [PubMed: 2229071]

6. Benne R et al. (1986) Major transcript of the frameshifted coxII gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA. Cell 46, 819–826 [PubMed: 3019552]
7. Gray MW et al. (2010) Cell biology. Irremediable complexity? Science 330, 920–921 [PubMed: 21071654]
8. Ochsenreiter T et al. (2008) Alternative mRNA editing in trypanosomes is extensive and may contribute to mitochondrial protein diversity. PLoS ONE 3, e1566 [PubMed: 18270563]
9. Ochsenreiter T et al. (2007) KISS: the kinetoplastid RNA editing sequence search tool. RNA 13, 1–4 [PubMed: 17123956]
10. Kirby LE et al. (2016) Analysis of the *Trypanosoma brucei* EATRO 164 bloodstream guide RNA transcriptome. PLoS Negl. Trop. Dis 10, e0004793 [PubMed: 27399202]
11. Koslowsky D et al. (2014) The insect-phase gRNA transcriptome in *Trypanosoma brucei*. Nucleic Acids Res. 42, 1873–1886 [PubMed: 24174546]
12. Aphasizhev R and Aphasizheva I (2014) Mitochondrial RNA editing in trypanosomes: Small RNAs in control. Biochimie 100, 125–131 [PubMed: 24440637]
13. Aphasizhev R and Aphasizheva I (2011) Uridine insertion/deletion mRNA editing in trypanosomes: a playground for RNA-guided information transfer. Wiley Interdiscip. Rev. RNA 2, 669–685 [PubMed: 21823228]
14. Hashimi H et al. (2013) Dual core processing: MRB1 is an emerging kinetoplast RNA editing complex. Trends Parasitol. 29, 91–99 [PubMed: 23305619]
15. Read LK et al. (2016) Trypanosome RNA editing: the complexity of getting U in and taking U out. Wiley Interdiscip. Rev. RNA 7, 33–51 [PubMed: 26522170]
16. Goringer HU (2012) 'Gestalt,' composition and function of the *Trypanosoma brucei* editosome. Annu. Rev. Microbiol 66, 65–82 [PubMed: 22994488]
17. Cruz-Reyes J et al. (2018) Dynamic RNA holo-editosomes with subcomplex variants: Insights into the control of trypanosome editing. Wiley Interdiscip. Rev. RNA 9, e1502 [PubMed: 30101566]
18. Zimmer SL et al. (2018) High throughput sequencing revolution reveals conserved fundamentals of U-indel editing. Wiley Interdiscip. Rev. RNA 9, e1487
19. Sement FM et al. (2018) Transcription initiation defines kinetoplast RNA boundaries. Proc. Natl. Acad. Sci. U. S. A 115, E10323–E10332 [PubMed: 30333188]
20. Suematsu T et al. (2016) Antisense transcripts delimit exonucleolytic activity of the mitochondrial 3' processome to generate guide RNAs. Mol. Cell 61, 364–378 [PubMed: 26833087]
21. Aphasizheva I and Aphasizhev R (2010) RET1-catalyzed uridylylation shapes the mitochondrial transcriptome in *Trypanosoma brucei*. Mol. Cell. Biol 30, 1555–1567 [PubMed: 20086102]
22. Mattiacio JL and Read LK (2008) Roles for TbDSS-1 in RNA surveillance and decay of maturation by-products from the 12S rRNA locus. Nucleic Acids Res. 36, 319–329 [PubMed: 18032430]
23. Ryan CM and Read LK (2005) UTP-dependent turnover of *Trypanosoma brucei* mitochondrial mRNA requires UTP polymerization and involves the RET1 TUTase. RNA 11, 763–773 [PubMed: 15811918]
24. Kao CY and Read LK (2005) Opposing effects of polyadenylation on the stability of edited and unedited mitochondrial RNAs in *Trypanosoma brucei*. Mol. Cell. Biol 25, 1634–1644 [PubMed: 15713623]
25. Zhang L et al. (2017) PPR polyadenylation factor defines mitochondrial mRNA identity and stability in trypanosomes. EMBOJ. 36, 2435–2454
26. Aphasizheva I et al. (2011) Pentatricopeptide repeat proteins stimulate mRNA adenylation/uridylation to activate mitochondrial translation in trypanosomes. Mol. Cell 42, 106–117 [PubMed: 21474072]
27. Etheridge RD et al. (2008) 3' adenylation determines mRNA abundance and monitors completion of RNA editing in *T. brucei mitochondria*. EMBO J. 27, 1596–1608 [PubMed: 18464794]
28. Mesitov MV et al. (2019) Pentatricopeptide repeat poly(A) binding protein KPAF4 stabilizes mitochondrial mRNAs in *Trypanosoma brucei*. Nat. Commun 10, 146 [PubMed: 30635574]

29. Aphasizheva I et al. (2016) Ribosome-associated pentatricopeptide repeat proteins function as translational activators in mitochondria of trypanosomes. Mol. Microbiol 99, 1043–1058 [PubMed: 26713541]
30. Ridlon L et al. (2013) The importance of the 45S ribosomal small subunit-related complex for mitochondrial translation in *Trypanosoma brucei*. J. Biol. Chem 288, 32963–32978 [PubMed: 24089529]
31. Saurer M et al. (2019) Mitoribosomal small subunit biogenesis in trypanosomes involves an extensive assembly machinery. Science 365, 1144–1149 [PubMed: 31515389]
32. Stuart KD et al. (2005) Complex management: RNA editing in trypanosomes. Trends Biochem. Sci 30, 97–105 [PubMed: 15691655]
33. Simpson L et al. (2010) Guide to the nomenclature of kinetoplastid RNA editing: a proposal. Protist 161, 2–6 [PubMed: 19945343]
34. Read LK et al. (1992) Extensive editing of both processed and preprocessed maxicircle CR6 transcripts in *Trypanosoma brucei*. J. Biol. Chem 267, 1123–1128 [PubMed: 1730639]
35. Koslowsky DJ and Yahampath G (1997) Mitochondrial mRNA 3' cleavage polyadenylation and RNA editing in *Trypanosoma brucei* are independent events. Mol. Biochem. Parasitol 90, 81–94 [PubMed: 9497034]
36. Michelotti EF et al. (1992) *Trypanosoma brucei* mitochondrial ribosomal RNA synthesis, processing and developmentally regulated expression. Mol. Biochem. Parasitol 54, 31–42 [PubMed: 1381496]
37. Carnes J et al. (2015) Bloodstream form *Trypanosoma brucei* do not require mRPN1 for gRNA processing. RNA 21, 28–35 [PubMed: 25404564]
38. Adler BK et al. (1991) Modification of *Trypanosoma brucei* mitochondrial rRNA by posttranscriptional 3' polyuridine tail formation. Mol. Cell. Biol 11, 5878–5884 [PubMed: 1719373]
39. Bhat GJ et al. (1991) The two ATPase 6 mRNAs of *Leishmania tarentolae* differ at their 3' ends. Mol. Biochem. Parasitol 48, 139–150 [PubMed: 1837067]
40. Blum B et al. (1990) A model for RNA editing in kinetoplastid mitochondria: 'Guide' RNA molecules transcribed from maxicircle DNA provide the edited information. Cell 60, 189–198 [PubMed: 1688737]
41. Madina BR et al. (2011) Guide RNA biogenesis involves a novel RNase III family endoribonuclease in *Trypanosoma brucei*. RNA 17, 1821–1830 [PubMed: 21810935]
42. Schnaufer A et al. (2001) An RNA ligase essential for RNA editing and survival of the bloodstream form of *Trypanosoma brucei*. Science 291, 2159–2161 [PubMed: 11251122]
43. Aphasizhev R et al. (2003) A tale of two TUTases. Proc. Natl. Acad. Sci. U. S. A 100, 10617–10622 [PubMed: 12954983]
44. Aphasizhev R et al. (2002) Trypanosome mitochondrial 3' terminal uridylyl transferase (TUTase): the key enzyme in U-insertion/deletion RNA editing. Cell 108, 637–648 [PubMed: 11893335]
45. Aphasizheva I et al. (2004) RNA-editing terminal uridylyl transferase 1: identification of functional domains by mutational analysis. J. Biol. Chem 279, 24123–24130 [PubMed: 15060068]
46. Aphasizheva I et al. (2014) RNA binding and core complexes constitute the U-insertion/deletion editosome. Mol. Cell. Biol 34, 4329–4342 [PubMed: 25225332]
47. Aphasizhev R et al. (2016) Constructive edge of uridylation-induced RNA degradation. RNA Biol. 13, 1078–1083 [PubMed: 27715485]
48. Menezes MR et al. (2018) 3' RNA uridylation in epitranscriptomics, gene regulation, and disease. Front. Mol. Biosci 5, 61 [PubMed: 30057901]
49. Jasmer D and Stuart K (1986) Sequence organization in African trypanosome minicircles is defined by 18 base pair inverted repeats. Mol. Biochem. Parasitol 18, 321–332 [PubMed: 3960057]
50. Pollard VW et al. (1990) Organization of minicircle genes for guide RNAs in *Trypanosoma brucei*. Cell 63, 783–790 [PubMed: 2171782]

51. Cooper S et al. (2019) Assembly and annotation of the mitochondrial minicircle genome of a differentiation-competent strain of *Trypanosoma brucei*. Nucleic Acids Res. 47, 11304–11325 [PubMed: 31665448]
52. Simpson L et al. (2015) Comparison of the mitochondrial genomes and steady state transcriptomes of two strains of the trypanosomatid parasite, *Leishmania tarentolae*. PLoS Negl. Trop. Dis 9, e0003841 [PubMed: 26204118]
53. Simpson RM et al. (2016) High-throughput sequencing of partially edited trypanosome mRNAs reveals barriers to editing progression and evidence for alternative editing. RNA 22, 677–695 [PubMed: 26908922]
54. Chung CZ et al. (2017) Tipping the balance of RNA stability by 3' editing of the transcriptome. Biochim. Biophys. Acta Gen. Subj 1861, 2971–2979
55. Ryan CM et al. (2003) Polyadenylation regulates the stability of *Trypanosoma brucei* mitochondrial RNAs. J. Biol. Chem 278, 32753–32762 [PubMed: 12801929]
56. Small ID and Peeters N (2000) The PPR motif–ma TPR related motif prevalent in plant organellar proteins. Trends Biochem. Sci 25, 46–47 [PubMed: 10664580]
57. Shen C et al. (2016) Structural basis for specific single-stranded RNA recognition by designer pentatricopeptide repeat proteins. Nat. Commun 7, 11285 [PubMed: 27088764]
58. Barkan A and Small I (2014) Pentatricopeptide repeat proteins in plants. Annu. Rev. Plant Biol 65, 415–442 [PubMed: 24471833]
59. Pfalz J et al. (2009) Site-specific binding of a PPR protein defines and stabilizes 5' and 3' mRNA termini in chloroplasts. EMBO J. 28, 2042–2052 [PubMed: 19424177]
60. Cheng S et al. (2016) Redefining the structural motifs that determine RNA binding and RNA editing by pentatricopeptide repeat proteins in land plants. Plant J. 85, 532–547 [PubMed: 26764122]
61. Feagin JE et al. (1988) Creation of AUG initiation codons by addition of uridines within cytochrome b transcripts of kinetoplastids. PNAS 85, 539–543 [PubMed: 2448777]
62. Koslowsky DJ et al. (1990) The MURF3 gene of *T. brucei* contains multiple domains of extensive editing and is homologous to a subunit of NADH dehydrogenase. Cell 62, 901–911 [PubMed: 2393904]
63. Seiwert SD et al. (1996) Direct visualization of uridylate deletion in vitro suggests a mechanism for kinetoplastid RNA editing. Cell 84, 831–841 [PubMed: 8601307]
64. Kable ML et al. (1996) RNA editing: a mechanism for gRNAspecified uridylate insertion into precursor mRNA [see comments]. Science 273, 1189–1195 [PubMed: 8703045]
65. Seiwert SD and Stuart K (1994) RNA editing: transfer of genetic information from gRNA to precursor mRNA in vitro. Science 266, 114–117 [PubMed: 7524149]
66. Sturm NR and Simpson L (1990) Kinetoplast DNA minicircles encode guide RNAs for editing of cytochrome oxidase subunit III mRNA. Cell 61, 879–884 [PubMed: 1693097]
67. Golden DE and Hajduk SL (2005) The 3'-untranslated region of cytochrome oxidase II mRNA functions in RNA editing of African trypanosomes exclusively as a cis guide RNA. RNA 11, 29–37 [PubMed: 15574518]
68. Koslowsky DJ et al. (1991) Cycles of progressive realignment of gRNA with mRNA in RNA editing. Cell 67, 537–546 [PubMed: 1718605]
69. Simpson RM et al. (2017) Trypanosome RNA Editing Mediator Complex proteins have distinct functions in gRNA utilization. Nucleic Acids Res. 45, 7965–7983 [PubMed: 28535252]
70. Gerasimov ES et al. (2018) Trypanosomatid mitochondrial RNA editing: dramatically complex transcript repertoires revealed with a dedicated mapping tool. Nucleic Acids Res. 46,765–781 [PubMed: 29220521]
71. Maslov DA and Simpson L (1992) The polarity of editing within a multiple gRNA-mediated domain is due to formation of anchors for upstream gRNAs by downstream editing. Cell 70, 459–467 [PubMed: 1379519]
72. Igo RP Jr. et al. (2002) RNA sequence and base pairing effects on insertion editing in *Trypanosoma brucei*. Mol. Cell. Biol 22, 1567–1576 [PubMed: 11839822]

73. Blanc V et al. (1999) The mitochondrial RNA ligase from *Leishmania tarentolae* can join RNA molecules bridged by a complementary RNA. J. Biol. Chem 274, 24289–24296 [PubMed: 10446205]
74. Cruz-Reyes J et al. (2001) Trypanosome RNA editing: simple guide RNA features enhance U deletion 100-fold. Mol. Cell. Biol 21, 884–892 [PubMed: 11154275]
75. Aphasizhev R et al. (2003) Isolation of a U-insertion/deletion editing complex from Leishmania tarentolae mitochondria. EMBO J. 22, 913–924 [PubMed: 12574127]
76. Panigrahi AK et al. (2003) Identification of novel components of *Trypanosoma brucei* editosomes. RNA 9, 484–492 [PubMed: 12649499]
77. Golas MM et al. (2009) Snapshots of the RNA editing machine in trypanosomes captured at different assembly stages *in vivo*. EMBO J. 28, 766–778 [PubMed: 19197238]
78. Li F et al. (2009) Structure of the core editing complex (L-complex) involved in uridine insertion/deletion RNA editing in trypanosomatid mitochondria. Proc. Natl. Acad. Sci. U. S. A 106, 12306–12310 [PubMed: 19590014]
79. Rusche LN et al. (1997) Purification of a functional enzymatic editing complex from *Trypanosoma brucei* mitochondria. EMBO J. 16, 4069–4081 [PubMed: 9233816]
80. Guo X et al. (2012) KREPB6, KREPB7, and KREPB8 are important for editing endonuclease function in *Trypanosoma brucei*. RNA 18, 308–320 [PubMed: 22184461]
81. Carnes J et al. (2012) KREX2 is not essential for either procyclic or bloodstream form *Trypanosoma brucei*. PLoS ONE 7, e33405 [PubMed: 22438925]
82. Ernst NL et al. (2009) Differential functions of two editosome exoUases in *Trypanosoma brucei*. RNA 15, 947–957 [PubMed: 19318463]
83. Carnes J et al. (2008) RNA Editing in *Trypanosoma brucei* requires three different editosomes. Mol. Cell. Biol 28, 122–130 [PubMed: 17954557]
84. Carnes J et al. (2017) *In vivo* cleavage specificity of *Trypanosoma brucei* editosome endonucleases. Nucleic Acids Res. 45, 4667–4686 [PubMed: 28334821]
85. Macrae IJ and Doudna JA (2007) Ribonuclease revisited: structural insights into ribonuclease III family enzymes. Curr. Opin. Struct. Biol 17, 138–145 [PubMed: 17194582]
86. Carnes J et al. (2012) Mutational analysis of *Trypanosoma brucei* editosome proteins KREPB4 and KREPB5 reveals domains critical for function. RNA 18, 1897–1909 [PubMed: 22919050]
87. McDermott SM et al. (2019) Editosome RNase III domain interactions are essential for editing and differ between life cycle stages in *Trypanosoma brucei*. RNA 25, 1150–1163 [PubMed: 31171708]
88. McDermott SM and Stuart K (2017) The essential functions of KREPB4 are developmentally distinct and required for endonuclease association with editosomes. RNA 23, 1672–1684 [PubMed: 28802260]
89. McDermott SM et al. (2016) The Architecture of *Trypanosoma brucei* editosomes. Proc. Natl. Acad. Sci. U. S. A 113, E6476–E6485 [PubMed: 27708162]
90. Schnaufer A et al. (2003) Separate Insertion and deletion subcomplexes of the *Trypanosoma brucei* RNA editing complex. Mol. Cell 12, 307–319 [PubMed: 14536071]
91. Schnaufer A et al. (2010) A protein–protein interaction map of trypanosome ~20S editosomes. J. Biol. Chem 285, 5282–5295 [PubMed: 20018860]
92. Kang X et al. (2006) Reconstitution of full-round uridinedeletion RNA editing with three recombinant proteins. Proc. Natl. Acad. Sci. U. S. A 103, 13944–13949 [PubMed: 16963561]
93. Gao G et al. (2005) Functional complementation of *Trypanosoma brucei* RNA *in vitro* editing with recombinant RNA ligase. Proc. Natl. Acad. Sci. U. S. A 102, 4712–4717 [PubMed: 15781861]
94. Rogers K et al. (2007) Uridylate-specific 3’ 5’-exoribonucleases involved in uridylate-deletion RNA editing in trypanosomatid mitochondria. J. Biol. Chem 282, 29073–29080 [PubMed: 17699520]
95. Ringpis GE et al. (2010) Mechanism of U insertion RNA editing in trypanosome mitochondria: the bimodal TUTase activity of the core complex. J. Mol. Biol 399, 680–695 [PubMed: 20362585]
96. Ringpis GE et al. (2010) Mechanism of U-insertion RNA editing in trypanosome mitochondria: characterization of RET2 functional domains by mutational analysis. J. Mol. Biol 399, 696–706 [PubMed: 20417643]

97. Ernst NL et al. (2003) TbMP57 is a 3' terminal uridylyl transferase (TUTase) of the *Trypanosoma brucei* editosome. Mol. Cell 11, 1525–1536 [PubMed: 12820966]
98. Deng J et al. (2005) Structural basis for UTP specificity of RNA editing TUTases from *Trypanosoma brucei*. EMBO J. 24,4007–4017 [PubMed: 16281058]
99. Gao G and Simpson L (2003) Is the *Trypanosoma brucei* REL1 RNA ligase specific for U-deletion RNA editing, and is the REL2 RNA ligase specific for U-insertion editing? J. Biol. Chem 278, 27570–27574 [PubMed: 12748175]
100. Huang CE et al. (2001) Roles for ligases in the RNA editing complex of *Trypanosoma brucei*: band IV is needed for U-deletion and RNA repair. EMBO J. 20, 4694–4703 [PubMed: 11532934]
101. Pollard VW et al. (1992) Native mRNA editing complexes from *Trypanosoma brucei* mitochondria. EMBO J. 11, 4429–4438 [PubMed: 1330537]
102. Osato D et al. (2009) Uridine insertion/deletion RNA editing in trypanosomatid mitochondria: In search of the editosome. RNA 15, 1338–1344 [PubMed: 19447916]
103. Aphasizheva I and Aphasizhev R (2015) U-insertion/deletion mRNA-editing holoenzyme: definition in sight. Trends Parasitol. 13, 1078–1083
104. Panigrahi AK et al. (2007) Mitochondrial complexes in *Trypanosoma brucei*: a novel complex and a unique oxidoreductase complex. Mol. Cell Proteomics 7, 534–545 [PubMed: 18073385]
105. Weng J et al. (2008) Guide RNA-binding complex from mitochondria of trypanosomatids. Mol. Cell 32, 198–209 [PubMed: 18951088]
106. Hashimi H et al. (2009) Kinetoplastid guide RNA biogenesis is dependent on subunits of the mitochondrial RNA binding complex 1 and mitochondrial RNA polymerase. RNA 15, 588–599 [PubMed: 19228586]
107. Madina BR et al. (2014) Native mitochondrial RNA-binding complexes in kinetoplastid RNA editing differ in guide RNA composition. RNA 20, 1142–1152 [PubMed: 24865612]
108. Huang Z et al. (2015) Integrity of the core mitochondrial RNA binding complex 1 is vital for trypanosome RNA editing. RNA 21, 2088–2102 [PubMed: 26447184]
109. McAdams NM et al. (2019) MRB10130 is a RESC assembly factor that promotes kinetoplastid RNA editing initiation and progression. RNA 25, 1177–1191 [PubMed: 31221726]
110. McAdams NM et al. (2018) MRB7260 is essential for productive protein–RNA interactions within the RNA editing substrate binding complex during trypanosome RNA editing. RNA 24, 540–556 [PubMed: 29330168]
111. Ammerman ML et al. (2010) TbRGG2 facilitates kinetoplastid RNA editing initiation and progression past intrinsic pause sites. RNA 16, 2239–2251 [PubMed: 20855539]
112. Dixit S et al. (2017) Differential binding of mitochondrial transcripts by MRB8170 and MRB4160 regulates distinct editing fates of mitochondrial mRNA in trypanosomes. mBio 8, e02288–16 [PubMed: 28143982]
113. Ammerman ML et al. (2012) Architecture of the trypanosome RNA editing accessory complex, MRB1. Nucleic Acids Res. 40, 5637–5650 [PubMed: 22396527]
114. Aphasizhev R et al. (2003) A 100-kD complex of two RNA binding proteins from mitochondria of Leishmania tarentolae catalyzes RNA annealing and interacts with several RNA editing components. RNA 9, 62–76 [PubMed: 12554877]
115. Nikpour N and Salavati R (2019) The RNA binding activity of the first identified trypanosome protein with Z-DNA-binding domains. Sci. Rep 9, 5904 [PubMed: 30976048]
116. Kumar V et al. (2019) Protein features for assembly of the RNA editing helicase 2 subcomplex (REH2C) in trypanosome holo-editosomes. PLoS One 14, e0211525 [PubMed: 31034523]
117. Madina BR et al. (2015) Native variants of the MRB1 complex exhibit specialized functions in kinetoplastid RNA editing. PLoS ONE 10, e0123441 [PubMed: 25928631]
118. Hernandez A et al. (2010) REH2 RNA helicase in kinetoplastid mitochondria: ribonucleoprotein complexes and essential motifs for unwinding and guide RNA (gRNA) binding. J. Biol. Chem 285, 1220–1228 [PubMed: 19850921]
119. Kumar V et al. (2016) REH2C Helicase and GRBC subcomplexes may base pair through mRNA and small guide RNA in kinetoplastid editosomes. J. Biol. Chem 291, 5753–5764 [PubMed: 26769962]

120. Aphasizheva I et al. (2009) Novel TUTase associates with an editosome-like complex in mitochondria of *Trypanosoma brucei*. RNA 15, 1322–1337 [PubMed: 19465686]
121. Stagno J et al. (2010) Structure of the mitochondrial editosome-like complex associated TUTase 1 reveals divergent mechanisms of UTP selection and domain organization. J. Mol. Biol 399, 464–475 [PubMed: 20403364]
122. Kao CY and Read LK (2007) Targeted depletion of a mitochondrial nucleotidyltransferase suggests the presence of multiple enzymes that polymerize mRNA 3' tails in *Trypanosoma brucei* mitochondria. Mol. Biochem. Parasitol 154, 158–169 [PubMed: 17543398]
123. Missel A et al. (1997) Disruption of a gene encoding a novel mitochondrial DEAD-box protein in *Trypanosoma brucei* affects edited mRNAs. Mol. Cell. Biol 17, 4895–4903 [PubMed: 9271369]
124. Li F et al. (2011) Trypanosome REH1 is an RNA helicase involved with the 3'−5' polarity of multiple gRNA-guided uridine insertion/deletion RNA editing. Proc. Natl. Acad. Sci. U. S. A 108, 3542–3547 [PubMed: 21321231]
125. Koller J et al. (1997) *Trypanosoma brucei* gBP21. An arginine rich mitochondrial protein that binds to guide RNA with high affinity. J. Biol. Chem 272, 3749–3757 [PubMed: 9013632]
126. Blom D et al. (2001) Cloning and characterization of two guide RNA-binding proteins from mitochondria of Crithidia fasciculata: gBP27, a novel protein, and gBP29, the orthologue of *Trypanosoma brucei* gBP21. Nucleic Acids Res. 29, 2950–2962 [PubMed: 11452020]
127. Zikova A et al. (2008) Structure and function of the native and recombinant mitochondrial MRP1/MRP2 complex from *Trypanosoma brucei*. Int. J. Parasitol 38, 901–912 [PubMed: 18295767]
128. Schumacher MA et al. (2006) Crystal structures of *T. brucei*MRP1/MRP2 guide-RNA binding complex reveal RNA matchmaking mechanism. Cell 126, 701–711 [PubMed: 16923390]
129. Muller UF and Goringer HU (2002) Mechanism of the gBP21-mediated RNA/RNA annealing reaction: matchmaking and charge reduction. Nucleic Acids Res. 30, 447–455 [PubMed: 11788706]
130. Muller UF et al. (2001) Annealing of RNA editing substrates facilitated by guide RNA-binding protein gBP21. EMBO J. 20, 1394–1404 [PubMed: 11250905]
131. Tylec BL et al. (2019) Intrinsic and regulated properties of minimally edited trypanosome mRNAs. Nucleic Acids Res. 47, 3640–3657 [PubMed: 30698753]
132. Fisk JC et al. (2009) Distinct and overlapping functions of MRP1/2 and RBP16 in mitochondrial RNA metabolism. Mol. Cell. Biol 29, 5214–5225 [PubMed: 19620277]
133. Vondruskova E et al. (2005) RNA interference analyses suggest a transcript-specific regulatory role for mitochondrial RNA-binding proteins MRP1 and MRP2 in RNA editing and other RNA processing in *Trypanosoma brucei*. J. Biol. Chem 280, 2429–2438 [PubMed: 15504736]
134. Hayman ML and Read LK (1999) *Trypanosoma brucei* RBP16 is a mitochondrial Y-box family protein with guide RNA binding activity. J. Biol. Chem 274, 12067–12074 [PubMed: 10207031]
135. Ammerman ML et al. (2008) gRNA/pre-mRNA annealing and RNA chaperone activities of RBP16. RNA 14, 1069–1080 [PubMed: 18441045]
136. Miller MM et al. (2006) RBP16 stimulates trypanosome RNA editing *in vitro* at an early step in the editing reaction. RNA 12, 1292–1303 [PubMed: 16691000]
137. Pelletier M and Read LK (2003) RBP16 is a multifunctional gene regulatory protein involved in editing and stabilization of specific mitochondrial mRNAs in *Trypanosoma brucei*. RNA 9, 457–468 [PubMed: 12649497]
138. Miller MM and Read LK (2003) *Trypanosoma brucei*: functions of RBP16 cold shock and RGG domains in macromolecular interactions. Exp. Parasitol 105, 140–148 [PubMed: 14969691]
139. Vanhamme L et al. (1998) *Trypanosoma brucei* TBRGG1, a mitochondrial oligo(U)-binding protein that co-localizes with an in vitro RNA editing activity. J. Biol. Chem 273, 21825–21833 [PubMed: 9705321]
140. Hashimi H et al. (2008) TbRGG1, an essential protein involved in kinetoplastid RNA metabolism that is associated with a novel multiprotein complex. RNA 14, 970–980 [PubMed: 18369185]
141. McAdams NM et al. (2015) An arginine-glycine-rich RNA binding protein impacts the abundance of specific mRNAs in the mitochondria of *Trypanosoma brucei*. Eukaryot. Cell 14, 149–157 [PubMed: 25480938]

142. Shaw PL et al. (2015) Structures of the T. *brucei* kRNA editing factor MRB1590 reveal unique RNA-binding pore motif contained within an ABC-ATPase fold. Nucleic Acids Res. 43, 7096–7109 [PubMed: 26117548]
143. Madison-Antenucci S and Hajduk SL (2001) RNA editing-associated protein 1 is an RNA binding protein with specificity for preedited mRNA. Mol. Cell 7, 879–886 [PubMed: 11336710]
144. Madison-Antenucci S et al. (1998) Kinetoplastid RNA-editing associated protein 1 (REAP-1): a novel editing complex protein with repetitive domains. EMBO J. 17, 6368–6376 [PubMed: 9799244]
145. Hans J et al. (2007) RNA-editing-associated protein 1 null mutant reveals link to mitochondrial RNA stability. RNA 13, 881–889 [PubMed: 17416633]
146. Dixit S and Lukes J (2018) Combinatorial interplay of RNA binding proteins tunes levels of mitochondrial mRNA in trypanosomes. RNA 24, 1594–1606 [PubMed: 30120147]
147. Zimmer SL et al. (2011) A novel member of the RNase D exoribonuclease family functions in mitochondrial guide RNA metabolism in *Trypanosoma brucei*. J. Biol. Chem 286, 10329–10340 [PubMed: 21252235]
148. Taschner A et al. (2012) Nuclear RNase P of *Trypanosoma brucei*: a single protein in place of the multicomponent RNA protein complex. Cell Rep. 2, 19–25 [PubMed: 22840392]
149. Salavati R et al. (2001) Mitochondrial ribonuclease P activity of *Trypanosoma brucei*. Mol. Biochem. Parasitol 115, 109–117 [PubMed: 11377745]
150. Kapushoc ST et al. (2002) Differential localization of nuclear encoded tRNAs between the cytosol and mitochondrion in *Leishmania tarentolae*. RNA 8, 57–68 [PubMed: 11871660]
151. Kapushoc ST et al. (2000) End processing precedes mitochondrial importation and editing of tRNAs in *Leishmania tarentolae*. J. Biol. Chem 275, 37907–37914 [PubMed: 10993905]
152. Rajappa-Titu L et al. (2016) RNA Editing TUTase 1: structural foundation of substrate recognition, complex interactions and drug targeting. Nucleic Acids Res. 44, 10862–10878 [PubMed: 27744351]
153. Mattiacio JL and Read LK (2009) Evidence for a degradosome-like complex in the mitochondria of *Trypanosoma brucei*. FEBS Lett. 583, 2333–2338 [PubMed: 19540236]
154. Mingler MK et al. (2006) Identification of pentatricopeptide repeat proteins in *Trypanosoma brucei*. Mol. Biochem. Parasitol 150, 37–45 [PubMed: 16837079]
155. Pusnik M et al. (2007) Pentatricopeptide repeat proteins in *Trypanosoma brucei* function in mitochondrial ribosomes. Mol. Cell. Biol 27, 6876–6888 [PubMed: 17646387]
156. Trotter JR et al. (2005) A deletion site editing endonuclease in *Trypanosoma brucei*. Mol. Cell 20, 403–412 [PubMed: 16285922]
157. Carnes J et al. (2005) An essential RNase III insertion editing endonuclease in *Trypanosoma brucei*. Proc. Natl. Acad. Sci.U. S. A 102, 16614–16619 [PubMed: 16269544]
158. Drozdz M et al. (2002) TbMP81 is required for RNA editing in *Trypanosoma brucei*. EMBO J. 21, 1791–1799 [PubMed: 11927563]
159. McDermott SM et al. (2015) Differential editosome protein function between life cycle stages of *Trypanosoma brucei*. J. Biol. Chem 290, 24914–24931 [PubMed: 26304125]
160. Salavati R et al. (2006) KREPA4, an RNA binding protein es- sential for editosome integrity and survival of *Trypanosoma brucei*. RNA 12, 819–831 [PubMed: 16601201]
161. Law JA et al. (2007) In *Trypanosoma brucei* RNA editing,TbMP18 (band VII) is critical for editosome integrity and for both insertional and deletional cleavages. Mol. Cell. Biol 27, 777–787 [PubMed: 17101787]
162. Tarun SZ Jr. et al. (2008) KREPA6 is an RNA-binding protein essential for editosome integrity and survival of *Trypanosoma brucei*. RNA 14, 347–358 [PubMed: 18065716]
163. McDermott SM et al. (2015) Identification by random mutagenesis of functional domains in KREPB5 that differentially af- fect RNA editing between life cycle stages of *Trypanosoma brucei*. Mol. Cell. Biol 35, 3945–3961 [PubMed: 26370513]
164. Carnes J et al. (2018) RNase III domain of KREPB9 and KREPB10 association with editosomes in *Trypanosoma brucei*. mSphere 3, e00585–17 [PubMed: 29359194]

165. Acestor N et al. (2009) The MRB1 complex functions in kinetoplastid RNA processing. RNA 15, 277–286 [PubMed: 19096045]

166. Ammerman ML et al. (2013) A core MRB1 complex component is indispensable for RNA editing in insect and human infective stages of *Trypanosoma brucei*. PLoS ONE 8, e78015 [PubMed: 24250748]

167. Ammerman ML et al. (2011) MRB3010 is a core component of the MRB1 complex that facilitates an early step of the kinetoplastid RNA editing process. RNA 17, 865–877 [PubMed: 21451155]

168. Kafkova L et al. (2012) Functional characterization of two paralogs that are novel RNA binding proteins influencing mitochondrial transcripts of *Trypanosoma brucei*. RNA 18, 1846–1861 [PubMed: 22898985]

169. Fisk JC et al. (2008) TbRGG2, an essential RNA editing accessory factor in two *Trypanosoma brucei life cycle stages*. J. Biol. Chem 283, 23016–23025 [PubMed: 18583347]

170. Foda BM et al. (2012) Multifunctional G-rich and RRM containing domains of TbRGG2 perform separate yet essential functions in trypanosome RNA editing. Eukaryot. Cell 11, 1119–1131 [PubMed: 22798390]

171. Travis B et al. (2019) The RRM of the kRNA-editing protein TbRGG2 uses multiple surfaces to bind and remodel RNA. Nucleic Acids Res. 47, 2130–2142 [PubMed: 30544166]

Highlights

Mitochondrial RNA processing events in kinetoplastid protists include 5′ modification, 3′–5′ degradation, internal sequence changes by U-insertion/deletion mRNA editing, and nontemplated 3′ extensions.

The specificity of mRNA editing is dictated by gRNAs while 5′ modifications and 3′ extensions are controlled by diverse pentatricopeptide repeat (PPR) RNA-binding factors.

Antisense transcription plays a central role in delimiting 3′ termini of mature RNAs.

Macromolecular protein and ribonucleoprotein (RNP) complexes and auxiliary factors involved in these processes have been identified and characterized to varying degrees. This review discusses recent developments and introduces a consensus nomenclature for mitochondrial RNA-processing complexes and factors in *Trypanosoma brucei*.

Outstanding Questions

Mitochondrial mRNA, gRNA, and rRNA genes are transcribed as 3′ extended precursors. What is the structure and positions of maxicircle promoters and terminators, and the precise role of antisense transcripts in delimiting mature RNA boundaries?

Are transcription complexes acting on maxicircle and minicircle genomes the same or different?

What is the timing and mutual dependence of RNA synthesis and processing events?

Most RESC subunits lack recognizable motifs but are essential for editing and cell viability. What are their specific functions?

What is the mechanism of 5′ to 3′ communication in mRNA biogenesis and function?

How does the mitochondrial ribosome select fully edited mRNAs and recognize the correct reading frame?

Are alternatively edited mRNAs translated into proteins with distinct functions?

How are stage-specific mitochondrial RNA-processing patterns coordinated with, or determined by, nuclear gene activity?

What is the role of the mitochondrial translation apparatus in stage-specific gene expression?

Do mitochondrial ribosomal proteins preassemble independently of ribosomal RNA?

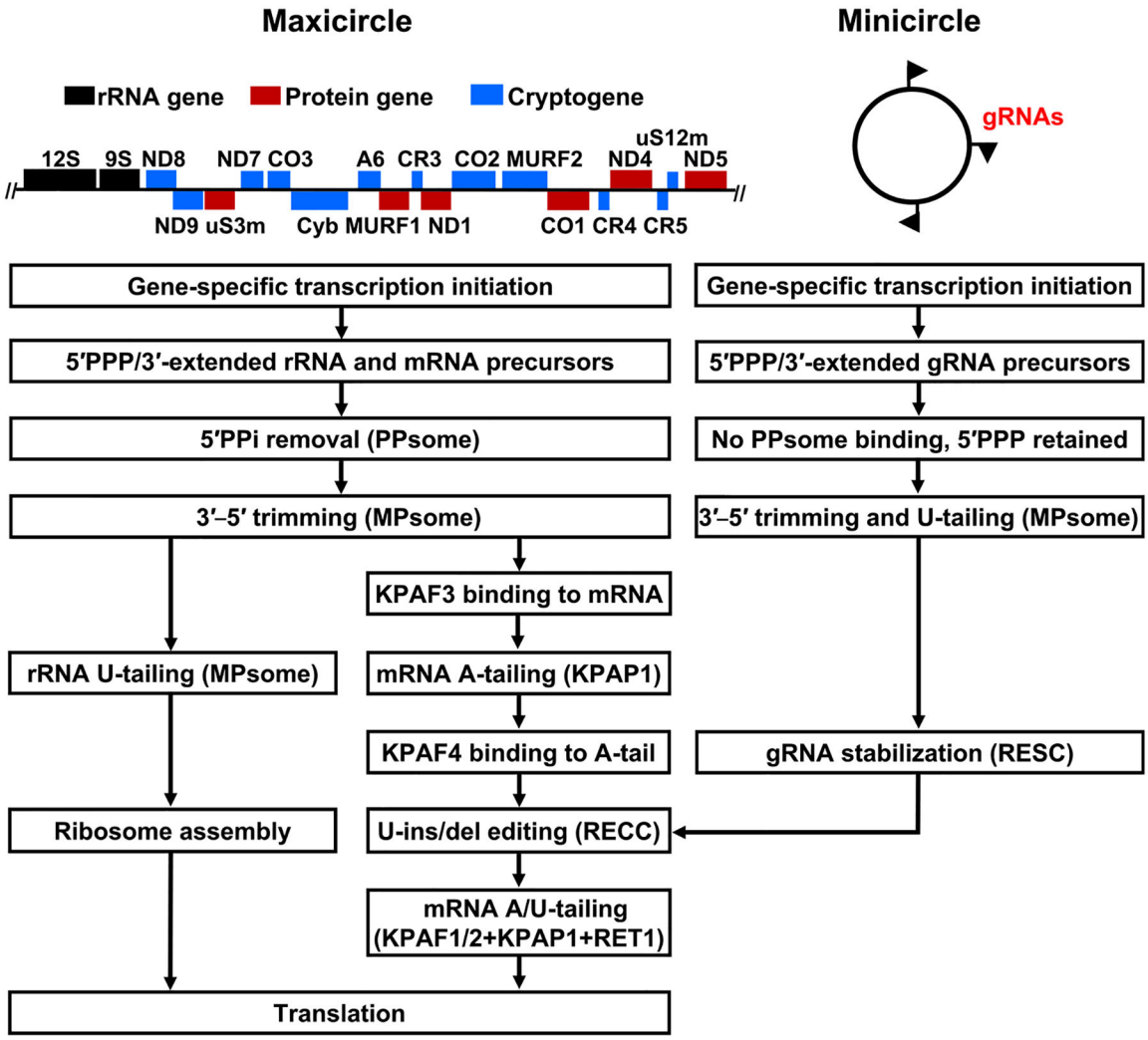

Figure 1. Schematic Diagram of Mitochondrial RNA Processing in *Trypanosoma brucei*.

The flow of processing reactions does not imply an experimentally established timing of these events. For example, the rRNA assembly into the ribosome or 5′ pyrophosphate removal from mRNA may occur cotranscriptionally. Likewise, the mRNA may be edited prior to completion of 3′–5′ trimming and 3′ adenylation. Abbreviations: MPsome, mitochondrial 3′ processome; PPi, inorganic pyrophosphate; PPsome, 5′ pyrophosphate processome; RECC, RNA-editing catalytic complex; RESC, RNA-editing substrate-binding complex.

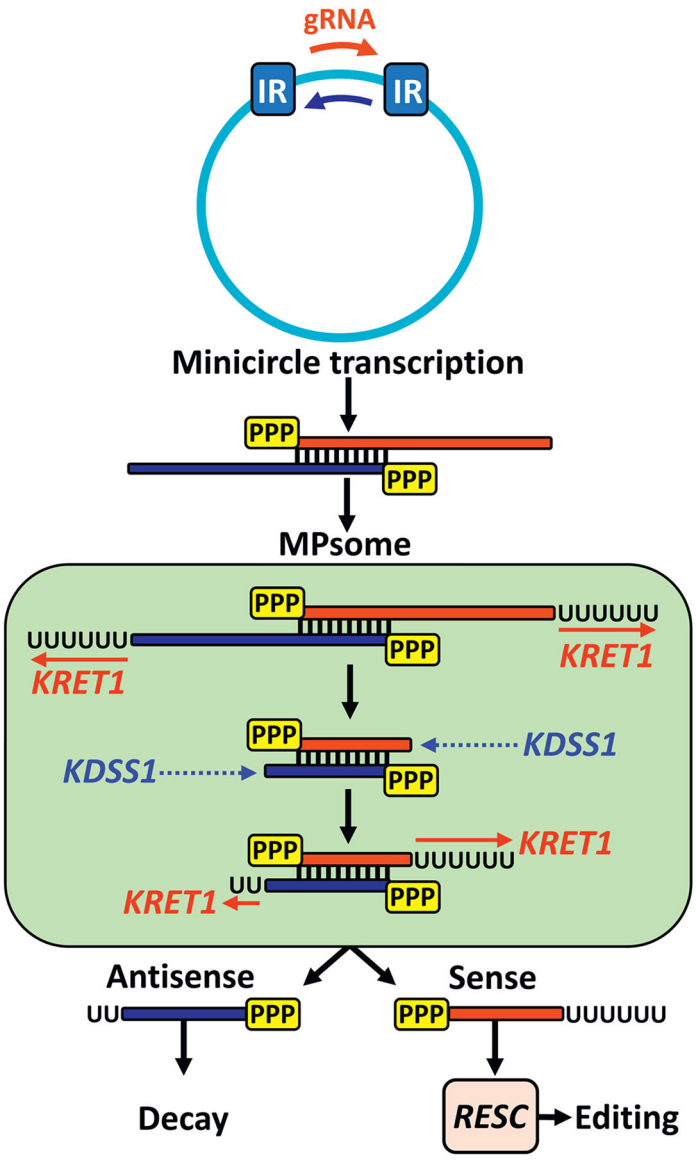

Figure 2. Guide RNA-Processing Model.

Bidirectional transcription of a guide (g)RNA gene from inverted repeats (IRs) generates overlapping sense and antisense precursors. In *Trypanosoma brucei*, a minicircle typically contains up to five gRNA genes. The mitochondrial 3′ processome (MPsome) catalyzes three sequential processing reactions: primary precursor uridylation, processive precursor degradation, and secondary uridylation of the mature gRNA. Primary uridylation by KRET1 stimulates hydrolytic activity of KDSS1. The MPsome stochastically pauses at 10–12 nt from sufficiently stable duplex regions, which provides a kinetic window for secondary uridylation. This step may disengage the MPsome from the duplex intermediate. Double-stranded RNA likely undergoes active unwinding before mature gRNA can be sequestered by the RESC complex and delivered into the editing pathway. Abbreviation: RESC, RNA-editing substrate-binding complex.

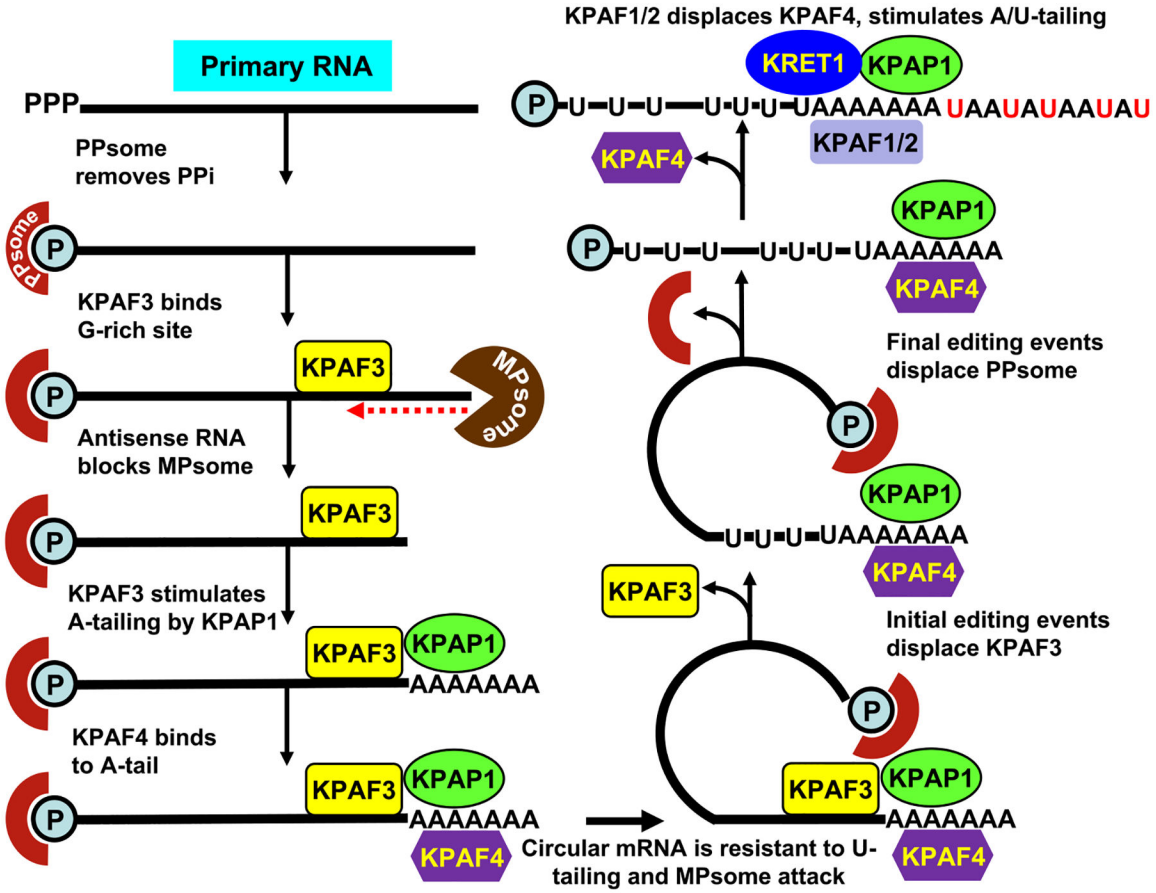

Figure 3. A Model for mRNA Quality Control by Pentatricopeptide Repeat (PPR) RNA-Binding Proteins.

We propose that mRNA stability, terminal modifications, and translational activation is largely determined by sequence-specific PPR RNA-binding proteins. Displacement of polyadenylation factor KPAF3 and the 5′ pyrophosphate processome (PPsome) subunit MERS2 by initiating and final editing events, respectively, appears to monitor editing progression and enables temporally separated modifications of the termini. The mRNA circularization is postulated to occur upon KPAF4 binding to a nascent A-tail and ensuing interaction with the PPsome. Displacement of the latter from the 5′ region by final editing events may provide access to KPAF1/2, which stimulates postediting A/U-tailing of fully edited mRNAs, leading to their translational activation. Abbreviation: MPsome, mitochondrial 3′ processome; PPi, inorganic pyrophosphate.

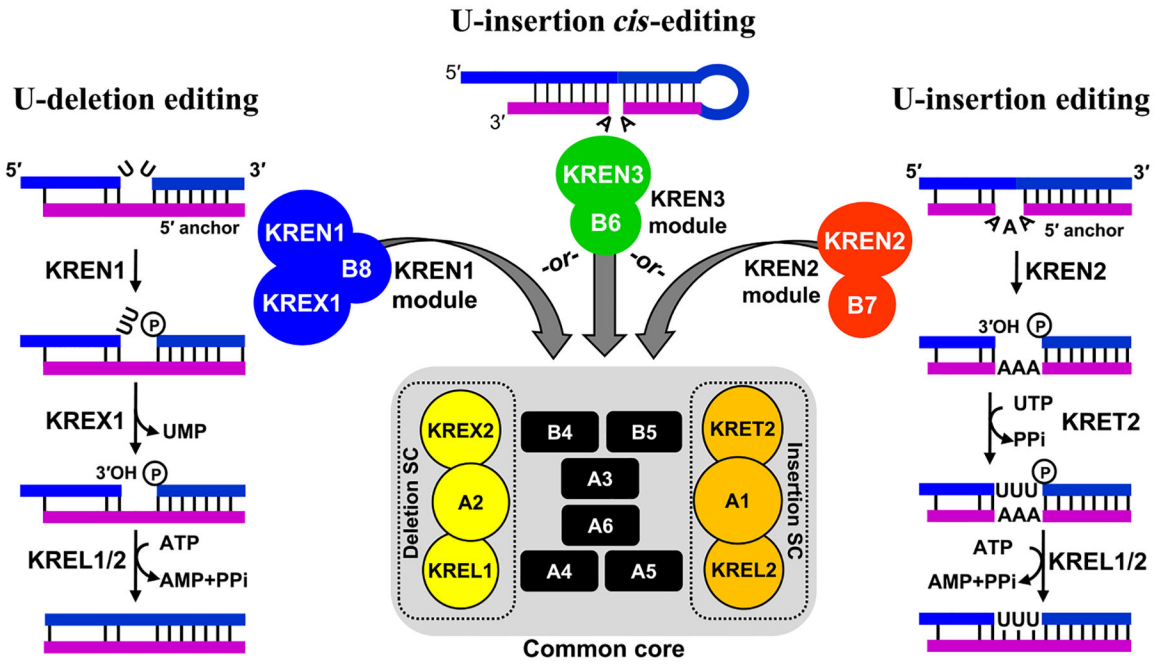

Figure 4. Schematic Diagram of RNA-Editing Catalytic Complex (RECC) Isoforms and Editing Reactions.

Trans-guided insertion and deletion, and *cis*-guided insertion substrates are juxtaposed with corresponding endonuclease modules and 12 common core proteins, with catalytic pathways outlined. Abbreviations: 5′ anchor, 5′ part of the guideRNA that hybridizes with pre-edited mRNA; A, KREPA; B, KREPB; SC, subcomplex. See Table 1 for protein designations.

Table 1.

Proposed Nomenclature of Mitochondrial RNA-Processing Complexes and Factors

Legacy		Assigned	Function	Motifs	TriTryp ID[a]	Refs
Nucleolytic processing: mitochondrial 3′ processome (MPsome)						
RET1	KRET1	KRET1	TUTase, 3′ uridylation of primary and mature RNAs	TUTase, PAP associated	Tb927.7.3950	[20,21,43,44,152]
KDSS1	KDSS1	KDSS1	3′–5′ exonuclease	RNB (ribonuclease II)	Tb927.9.7210	[20,22,153]
MPSS1		MPSS1			Tb927.11.9150	[20]
MPSS2		MPSS2			Tb927.10.9000	[20]
MPSS3		MPSS3			Tb927.3.2770	[20]
MPSS4		MPSS4			Tb927.10.6170	[20]
MPSS5		MPSS5			Tb927.9.4810	[20]
MPSS6		MPSS6			Tb927.6.2190	[20]
Modification of the 5′ end: pyrophosphohydrolase complex (PPsome)						
MERS1		MERS1	PPi removal from 5′ end	NUDIX hydrolase	Tb927.11.15640	[19,105,106]
MERS2		MERS2	Targets MERS1 to RNA	PPR	Tb11.02.5120	[19]
Modification of the 3′ end: kinetoplast polyadenylation complex (KPAC)						
KPAP1		KPAP1	Major poly(A) polymerase	NT/TUTase, PAP associated	Tb927.11.7960	[27]
KPAF1	PPR1	KPAF1	mRNA A/U-tailing	PPR	Tb927.2.3180	[26,154,155]
KPAF2		KPAF2	mRNA A/U-tailing	PPR	Tb927.11.14380	[26]
KPAF3		KPAF3	mRNA stabilization/A-tailing	PPR	Tb927.9.12770	[25]
KPAF4		KPAF4	Poly(A) binding protein	PPR	Tb927.10.10160	[28]
U-insertion/deletion mRNA editing: RNA-editing catalytic complex (RECC)						
REN1	KREPB1	KREN1	U-deletion endonuclease	RNase III, PUF, ZF-C2H2	Tb927.1.1690	[76,156]
REN2	KREPB3	KREN2	U-insertion endonuclease	RNase III, PUF, ZF-C2H2	Tb927.10.5440	[76,157]
REN3	KREPB2	KREN3	U-insertion endonuclease	RNase III, PUF, ZF-C2H2	Tb927.10.5320	[75,76,83]
REX1	KREX1	KREX1	3′−5′ U-specific exonuclease	Exo/endo/phos (EEP)	Tb927.7.1070	[75,76,82]
REX2	KREX2	KREX2	3′−5′ U-specific exonuclease	Exo/endo/phos (EEP)	Tb927.10.3570	[75,76,82]
RET2	KRET2	KRET2	U-insertion TUTase	TUTase, PAP associated	Tb927.7.1550	[43,75,76,97]
REL1	KREL1	KREL1	RNA ligase (U-deletion)	RNA lig/RNL2	Tb927.9.4360	[42,75,76,90]

Legacy		Assigned	Function	Motifs	TriTryp ID[a]	Refs
REL2	KREL2	KREL2	RNA ligase (U-insertion)	RNA lig/RNL2	Tb927.1.3030	[75,76,90]
MP81	KREPA1	KREPA1		ZF-C2H2, OB fold	Tb927.2.2470	[75,76,158]
MP63	KREPA2	KREPA2		ZF-C2H2, OB fold	Tb927.10.8210	[75]
MP42	KREPA3	KREPA3		ZF-C2H2, OB fold	Tb927.8.620	[75,76,159]
MP24	KREPA4	KREPA4		OB fold	Tb927.10.5110	[75,76,160]
MP19	KREPA5	KREPA5		OB fold	Tb927.8.680	[75,76]
MP18	KREPA6	KREPA6		OB fold	Tb927.10.5120	[75,76,161,162]
MP46	KREPB4	KREPB4		RNase III, PUF, ZF-C2H2	Tb927.11.2990	[75,76,88]
MP44	KREPB5	KREPB5		RNase III, PUF, ZF-C2H2	Tb927.11.940	[163]
MP49	KREPB6	KREPB6		RNase III, ZF-C2H2	Tb927.3.3990	[75,76,80,87]
MP47	KREPB7	KREPB7		RNase III, ZF-C2H2	Tb927.9.5630	[80,87]
MP41	KREPB8	KREPB8		RNase III, ZF-C2H2	Tb927.8.5690	[80,87]
	KREPB9	KREPB9		RNase III, ZF-C2H2	Tb927.9.4440	[164]
	KREPB10	KREPB10		RNase III, ZF-C2H2	Tb927.8.5700	[164]
U-insertion/deletion mRNA editing: RNA-editing substrate-binding complex (RESC)						
GRBC1	GAP2	RESC1	gRNA binding/ stabilization		Tb927.7.2570	[105,106]
GRBC2	GAP1	RESC2	gRNA binding/ stabilization		Tb927.2.3800	[105,106]
GRBC3	MRB8620	RESC3			Tb927.11.16860	[46,108,113]
GRBC4	MRB5390	RESC4			Tb11.02.5390b	[46,113,165]
GRBC5	MRB11870	RESC5			Tb927.10.11870	[46,113,166]
GRBC6	MRB3010	RESC6			Tb927.5.3010	[46,107,113,117,167]
GRBC7	MRB0880	RESC7			Tb927.11.9140	[46,113]
REMC1	MRB10130	RESC8	RNA binding	ARM/HEAT repeats	Tb927.10.10130	[46,104,109,113]
REMC2	MRB1860	RESC9			Tb927.2.1860	[46,113]
REMC3	MRB800	RESC10			Tb927.7.800	[46,113]
REMC4	MRB8180 MRB4150	RESC11A RESC11B	RNA binding		Tb927.8.8180 Tb927.4.4150	[46,69,113]
REMC5	MRB4160	RESC12	RNA binding		Tb927.4.4160	[46,112,113,168]
REMC5A	MRB8170	RESC12A	RNA binding		Tb927.8.8170	[46,69,112,113,168]
TbRGG2	TbRGG2	RESC13	RNA binding	RGG, RRM	Tb927.10.10830	[46,69,111,113,165,169–171]
	MRB7260	RESC14		PhyH	Tb927.9.7260	[110,113]

Legacy		Assigned	Function	Motifs	TriTryp ID[a]	Refs
PAMC1		RESC15			Tb927.1.1730	[46]
PAMC2		RESC16			Tb927.6.1200	[46]
PAMC3		RESC17			Tb927.10.1730	[46]
PAMC4		RESC18			Tb927.1.3010	[46]
MERS3	RBP7910	RESC19		Z-DNA binding	Tb927.10.7910	[19,115]
U-insertion/deletion mRNA editing: RNA-editing helicase 2 complex (REH2C)						
REH2		KREH2	RNA helicase, RNA binding	DEAH/RHA, HA2, DSRM, OB/NTP_binding	Tb927.4.1500	[104–107,116–119]
H2F1	MRB1680	KH2F1		ZF-C2H2	Tb927.6.1680	[118,119,165]
H2F2		KH2F2			Tb927.6.2140	[116,118,119]
Auxiliary factors						
MEAT1		MEAT1	RECC-like associated TUTase	TUTase, PAP associated	Tb927.1.1330	[120]
KPAP2		KPAP2	Putative poly(A) polymerase	NT/TUTase, PAP associated	Tb927.10.160	[122]
REH1	mHEL61	KREH1	RNA helicase	DEAD/DEAH box helicase	Tb927.11.8870	[123,124]
MRP1	gBP21	KMRP1	RNA binding		Tb927.11.1710	[114,125–133]
MRP2	gBP25	KMRP2	RNA binding		Tb927.11.13280	[114,127,128,131–133]
RGG1		KRGG1	RNA binding		Tb927.6.2230	[106,139,140]
RBP16		KRBP16	RNA binding	Cold-shock RNA binding	Tb927.11.7900	[135–138]
	MRB1590	KRBP72	RNA binding	ABC-like ATPase domain	Tb927.3.1590	[142]
TbRGG3	MRB1820	KRGG3			Tb927.3.1820	[113,141]
REAP-1		KREAP1	RNA binding		Tb927.10.9720	[143–145]
RND		KRND1	U-specific 3′–5′ exonuclease	RND, ZF-C2H2	Tb927.9.12720	[147]
PRORP2		KRNP1	RNase P	PRORP, PPR	Tb927.11.3010	[148]
mRPN1		KRPN1	Endonuclease	RNase III	Tb927.11.8400	[37,41]

[a]Gene identification numbers refer to *Trypanosoma brucei* strain TREU927 predicted protein sequences (TriTrypDB, Release 45, September 5, 2019, https://tritrypdb.org/tritrypdb/).